Journal of Chromatography, 520 (1990) 339–347
Elsevier Science Publishers B.V., Amsterdam

CHROMSYMP. 1913

Determination of cannabinoid acids by high-performance liquid chromatography of their neutral derivatives formed by thermal decarboxylation

I. Study of the decarboxylation process in open reactors

T. VERESS
Technical University, Laboratory of Forensic Sciences, Egry J.1, H-1111 Budapest (Hungary)
J. I. SZANTO
Institute of Forensic Science, P.O.B. 314/4, H-1903 Budapest (Hungary)
and
L. LEISZTNER*
Technical University, Laboratory of Forensic Sciences, Egry J.1, H-1111 Budapest (Hungary)

ABSTRACT

Decarboxylation of cannabidiolic and tetrahydrocannabinolic acids was studied in open reactors in order to investigate the accuracy and reliability of the decarboxylation sample preparation process applied prior to indirect methods, which has been widely used for the determination of cannabinoid acids. The rate of the decarboxylation reaction was followed by the high-performance liquid chromatographic determination of the neutral cannabinoids formed. The effects of different parameters (temperature, solvents, sorbent phases, salts) on decarboxylation were investigated. Reliable results could only be obtained by the mathematical correction of data obtained from experiments in an open reactor.

INTRODUCTION

The cannabinoid acids are among the main constituents of hashish and marihuana. They have no biological activity, but they can easily undergo decomposition resulting in neutral cannabinoids, especially tetrahydrocannabinols, that have psychotropic effects [1]. Owing to the thermal lability of cannabinoid acids, indirect methods [2–6] have been widely used for their determination. These indirect methods are based on decarboxylation of the acids and subsequent high-performance liquid chromatographic (HPLC) determination of the neutral cannabinoids formed.

The decarboxylation of cannabinoid acids was investigated by Kanter *et al.* [6], who suggested decarboxylation in a closed reactor at 200°C for 3 min. Smith [7] applied 100°C for 60 min to decarboxylate cannabinoid acids. Turner and Mahlberg [8] heated the samples at 37 and 60°C for several hours in an open reactor. In a previous paper [9] we reported the evaporation of neutral cannabinoids during decarboxylation

0021-9673/90/$03.50 © 1990 Elsevier Science Publishers B.V.

in an open reactor even below 200°C, and in closed reactors the conversion of the decarboxylation was found to be dependent on the volume of the reactor and seemed to be affected by different additional factors.

In this work we aimed to investigate the decarboxylation process by performing the reaction on the glass surface of the reactor and on the surfaces of different adsorbents packed into reactor tubes and in different solvent phases, using open reactors. Another aim was to develop a mathematical correction method to eliminate the systematic errors caused by the time dependence of the decarboxylation of cannabinoid acids and the simultaneous evaporation of neutral cannabinoids formed during decarboxylation in either the presence or absence of solvent.

EXPERIMENTAL

Materials and equipment

The homogenization and grinding of marihuana samples were carried out in a Straume electric grinder (U.S.S.R.). Extraction was performed in a UW2 ultrasonic bath (Elektrymat, Dresden, G.D.R.). For filtration Whatman GF/D glass fibre and 2-μm pore-size filter tips (Supelco) were used.

The decarboxylation experiments were done in a Reacti-Therm heating module (Pierce) using Sep-Pak (Waters) or Samplex Si-100 (Bioseparation Gmk., Budapest, Hungary) sample clean-up columns. For the laboratory-packed reactors 20–40-mesh activated carbon (Aldrich) and Nuchar Attaclay (Supelco) sorbents were used. All of the salts used for the modification of silica were of analytical-reagent grade (Reanal, Budapest, Hungary).

The HPLC separation and data handling were performed on a Kontron (Zurich, Switzerland) HPLC System 400 liquid chromatograph with the following configuration: two Model 420 HPLC pumps, a Model 460 autosampler, a Model 480 column oven, a Model 430 rapid-scanning UV–VIS detector and an IBM/AT-compatible Model 450 data system.

The methanol, hexane and ethanol used for HPLC separations were of LiChrosolv grade (Merck). The water used in the mobile phase was doubly distilled water according to Gurkin and Ripphahn's method [10].

Thin-layer chromatographic (TLC) separation was carried out on silica gel 60 F_{254} plates (Merck) and developed in analytical-reagent grade toluene (Reanal).

The neutral cannabinoid standards, Δ^9-tetrahydrocannabinol (Δ^9-THC), Δ^8-tetrahydrocannabinol (Δ^8-THC), cannabinol (CBN) and cannabidiol (CBD), were obtained from the United Nations Division of Narcotic Drugs. The marihuana samples investigated were seized by Customs Offices.

For the extraction, pro.synth.-grade diethylene glycol (Laborchemie, G.D.R.), analytical-reagent grade ethylene glycol (Reanal), *n*-octanol (Interkémia, Budapest, Hungary), dioctyl phthalate (Janssen Chimica, Beerse, Belgium), Uvasol-grade dimethyl sulphoxide (Merck) and LiChrosolv-grade *n*-hexane (Merck) were applied.

Extraction

From air-dried powdered marihuana, 800 mg were weighed and extracted with 20 ml of different organic solvents (ethylene glycol, diethylene glycol, *n*-octanol, dioctyl phthalate, dimethyl sulphoxide or *n*-hexane) by sonication for 10 min. The

extract was filtered and aliquots of the filtrate were used for the decarboxylation experiments.

Decarboxylation on glass surface

A 0.5-ml volume of *n*-hexane marihuana extract was measured into a 2.5-ml glass test-tube (10 mm I.D.) made of normal sodium glass and the solvent was evaporated at ambient temperature by flushing with nitrogen. The test-tubes were placed in the heating module and thermostated at 80, 94, 106, 122 and 145°C for different times in the range 0–50 min. The residue was dissolved in 1 ml of *n*-hexane and analysed by HPLC.

Decarboxylation on different sorbent surfaces

The experiments were performed in Sep-Pak cartridges containing acidic, basic or neutral alumina and also in original and modified Samplex Si-100 sample clean-up columns containing silica and in laboratory-packed reactors.

The modification of silica was performed by impregnation using methanol saturated with different salts at ambient temperature. To the silica 2 ml of methanolic solution were applied and the sorbent was activated at 109°C for 2 h.

For laboratory-packed reactors activated carbon and Nuchar Attaclay sorbents were used. A 1.5-cm^3 amount of each sorbent was filled into a 2-cm^3 polyethylene syringe.

Volumes of 0.5 or 1.5 ml of *n*-hexane extracts were applied to the reactor tubes packed with unmodified or modified sorbents, respectively. The decarboxylation was performed at 145°C for 10 min. After the decarboxylation, cannabinoids were eluted with 5 ml of methanol and the eluate was evaporated to dryness. The residue was dissolved in the same volume of *n*-hexane as used for the sample application.

Decarboxylation in solvent phase

From each of the extracts prepared with ethylene glycol, diethylene glycol, *n*-octanol, dioctyl phthalate or dimethyl sulphoxide, 0.2-ml volumes were measured into 2.5-ml glass test-tubes (10 mm I.D.), which were placed in the heating module and thermostated at different temperatures in the range 125–165°C for different times. The heated solutions were cooled to ambient temperature and diluted to 1.4 ml with the appropriate HPLC mobile phase.

TLC monitoring of the cannabinoid content of the effluents

A TLC method [11] was applied for rapid qualitative tracing of cannabinoids in order to monitor the cannabinoid content of the evolved gases during the decarboxylation on glass and sorbent surfaces and in the solvent phase, and also of effluents obtained by dissolving from sorbents after decarboxylation on sorbent surfaces. The cannabinoid content of the evolved gases was concentrated on the chromatographic sorbent layer by leading the gas stream directly onto the plate; the effluent solutions were applied by dropping with micropipettes.

HPLC separation

In order to ensure the compatibility of extracts with mobile phases, the determination of neutral cannabinoids was performed in the reversed- or normal-

phase mode depending on the solvent used for the sample preparation. In this manner the waste due to the redissolution could be eliminated.

With the reversed-phase system, the conditions were as follows: mobile phase, methanol–water (85:15); analytical column, Zorbax BP tm C_8 (25 cm × 4.6 mm I.D.); guard column, Brownlee RP-GU cartridge packed with LiChrosorb 10 RP-8 (3 cm × 4.6 mm I.D.); flow-rate, 1.2 ml/min; temperature, 60°C; detection, 220 nm; and injection, 20 μl.

In the normal-phase mode, the following conditions were applied: mobile phase, *n*-hexane–ethanol (97:3); analytical column, Ultrasil-NH_2, d_p = 10 μm (25 cm × 4.6 mm I.D.); guard column, Brownlee Amino cartridge packed with LiChrosorb 10 Amino (3 cm × 4.6 mm I.D.); flow-rate, 1.8 ml/min; temperature, 40°C; detection, 215 nm; and injection, 20 μl.

The determination of neutral cannabinoids was performed by the external standard method by measuring the peak heights. All the experiments were done triplicate with a relative standard deviation not greater than 5%.

RESULTS AND DISCUSSION

Two typical chromatograms obtained using the normal-phase system are shown in the Figs. 1 and 2. Fig. 1 shows the separation of a neutral cannabinoid test mixture. The Δ^8-THC isomer elutes first, followed by Δ^9-THC, CBD and CBN (the elution order in the reversed-phase mode is CBD, CBN, Δ^9-THC and Δ^8-THC, where the two isomers are also not totally resolved). Fig. 2 shows the chromatogram for a non-heated *n*-hexane marihuana extract, which does not contain any Δ^8-THC.

Decarboxylation on glass surface

Quantitative results obtained for Δ^9-THC are shown in Fig. 3. The results indicate that below 122°C the amount of THC increased even after heating for 50 min.

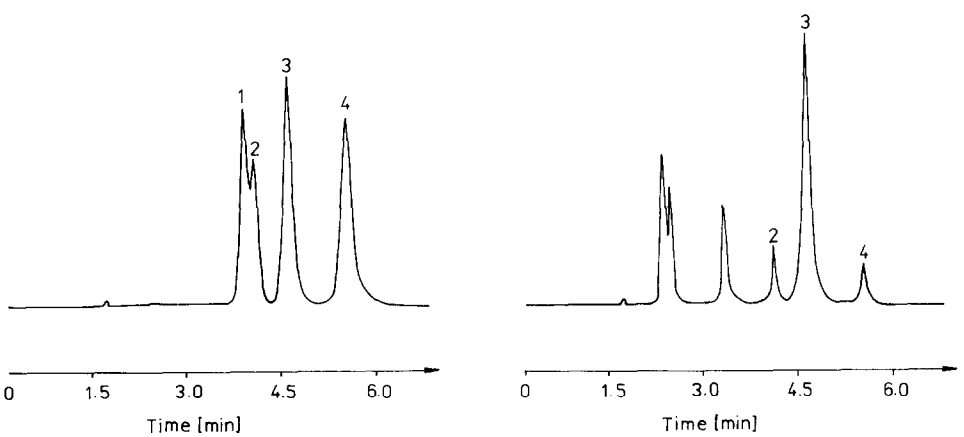

Fig. 1. Normal-phase HPLC separation of a neutral cannabinoid test mixture containing (1) Δ^8-THC, (2) Δ^9-THC, (3) CBD and (4) CBN. For details, see text.

Fig. 2. Typical separation of a non-heated *n*-hexane marihuana extract in the normal-phase mode. Peaks: 2 = Δ^9-THC; 3 = CBD; 4 = CBN. For details, see text.

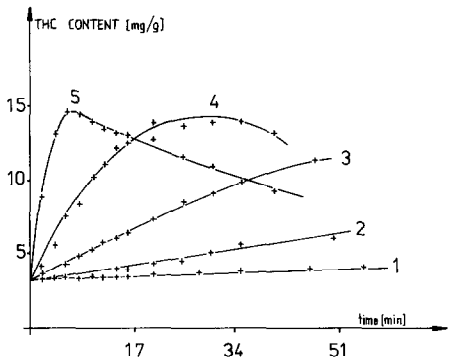

Fig. 3. Effect of heating time and temperature on the THC content of an *n*-hexane marihuana extract after heating on the glass surface in an open reactor. Curves: 1 = 80°C; 2 = 94°C; 3 = 106°C; 4 = 122°C; 5 = 145°C.

This illustrates the low velocity of the decarboxylation. Similar results were obtained for CBD. The decrease in the amount of cannabinoids observed at 122 and 145°C is caused by the evaporation, which was confirmed by TLC analysis of the evolved gases. Owing to the simultaneous evaporation during the decarboxylation, the total cannabinoid content could not be directly measured.

Calculation of cannabinoid acid content using a mathematical correction method

For the evaluation of the quantitative results obtained using the open reactor, a mathematical correction method was elaborated. The method takes into consideration the time dependence of the decarboxylation and the process causing waste. Using this method, the amount of cannabinoid acids could be calculated with elimination of the systematic errors caused by partial conversion and evaporation.

The decarboxylation of cannabinoid acids was considered to be a first-order reaction. The waste caused by the evaporation was approached by a virtual consecutive first-order reaction, which resulted in a decrease in neutral cannabinoids. Omitting the detailed derivation, in an open reactor under isothermal, isobaric conditions, the concentration (c) of a given neutral cannabinoid after a time t of heating can be expressed by

$$c = \frac{k_1 c_a [\exp(-k_1 t) - \exp(-k_2 t)] + c_n(k_2 - k_1) \exp(-k_1 t)}{(k_2 - k_1)} \quad (1)$$

where c_a and c_n are the concentrations of the given cannabinoid acid and neutral cannabinoid in the non-heated sample, respectively, and k_1 and k_2 are the velocity constants of the decarboxylation and the virtual consecutive reaction, respectively.

The data obtained from experiments in the open reactor, were evaluated according to eqn. 1 applying the least-squares method. The calculated results are given in Table I, together with the standard deviations of the residuals of the regression (s_{reg}). It can be seen that at 145°C the k_2 value for decarboxylation of THC acid is three times greater than that for CBD acid whereas the k_1 values are similar for both compounds. The c_a values calculated from the experimental data obtained by decarboxylation at

TABLE I

VELOCITY CONSTANTS (k_1, k_2) AND CONTENT OF CANNABINOID ACIDS (c_a) CALCULATED ACCORDING TO EQN. 1 (n = 13) AND CONTENT OF CANNABINOID ACIDS (c_a^*) OBTAINED BY HPLC WITH DECARBOXYLATION IN AN OPEN REACTOR WITHOUT SOLVENT

c_a^* = Possible maximum measured values of cannabinoid acids obtained by HPLC (for details, see text); d = percentage difference between c_a and c_a^*; n = number of data pairs used for the calculation.

Acid	T (°C)	k_1 (min^{-1})	k_2 (min^{-1})	s_{reg} (mg/g)	c_a (mg/g)	c_a^* (mg/g)	d (%)
CBD	80	0.001	0.000	1.09	324	34	89.5
	94	0.002	0.000	2.19	331	50	84.9
	106	0.006	0.000	4.36	313	114	63.6
	122	0.032	0.000	14.97	340	279	17.9
	145	0.170	0.007	14.36	327	309	5.5
THC	80	0.001	0.000	0.005	20.1	4.3	78.6
	94	0.003	0.000	0.034	20.4	6.4	68.6
	106	0.013	0.000	0.018	21.2	12.9	39.1
	122	0.060	0.010	0.072	20.1	15.8	21.4
	145	0.225	0.020	0.111	19.0	16.9	11.0

different temperatures show good agreement with each other. In order to demonstrate the necessity for correction, in Table I a comparison of amounts of acidic cannabinoids obtained by calculation and measurement is also shown. The cannabinoid acid measured contents (c_a^*) were determined as the maximum possible value of measurements obtained by a second-order curve fitting on the five greatest values corrected by the neutral cannabinoid content of the non-heated sample. The difference between the compared values are greater at lower temperatures, because the correction took into consideration the low velocity of the decarboxylation, whereas the directly measurable amounts are fairly low owing to the low reaction velocity.

Decarboxylation on different sorbent surfaces

TLC monitoring of the gases evolved during the decarboxylation on sorbent surfaces showed that evaporation of cannabinoids did not occur.

After decarboxylation on the surface of either activated carbon or Nuchar Attaclay sorbents, the cannabinoids were retained so strongly that none of them could be eluted with either methanol or with carbon disulphide. With acidic, basic and neutral alumina sorbents significantly lower cannabinoid contents were detected than with silica. After heating on silica more neutral cannabinoids were detected than with the non-heated samples, which indicated the decarboxylation of cannabinoid acids on silica.

The decarboxylation was studied on silica at 106 and 145°C as a function of the heating time. The results obtained at 145°C are shown in Fig. 4. The original concentration of Δ^9-THC and CBN in the non-heated sample is indicated at heating time zero. The sample investigated in these experiments did not originally contain CBD, Δ^8-THC and its acid derivative. On increasing the time of heating the Δ^8-THC isomer appeared and its increase could be detected, whereas the amount of Δ^9-THC

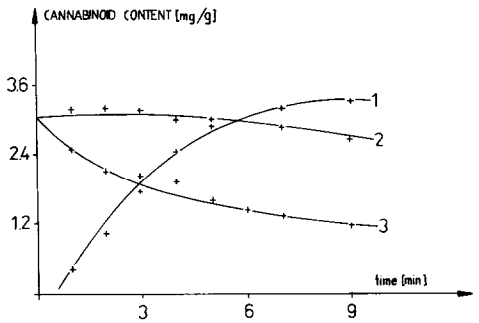

Fig. 4. Effect of heating time on the cannabinoid content of an *n*-hexanic marihuana extract after heating on the silica surface at 145°C for 10 min. Curves: 1 = Δ^8-THC; 3 = Δ^9-THC; 2 = CBN.

decreased. At 106°C the same effect was found but the amount of Δ^8-THC did not exceed that of Δ^9-THC during the 30-min interval investigated. During the experiments the amount of CBN decreased slightly.

Δ^9-THC reference compound was heated on silica under the same conditions as described above. Isomerization of Δ^9- to Δ^8-THC was found. Δ^8-THC was identified by comparison of its retention in HPLC and TLC systems with those of Δ^8-THC reference compound.

According to the isomerization of Δ^9-THC the change in concentration of the two THC isomers can be interpreted: the amount of Δ^9-THC decreases because the velocity of isomerization is greater than that of the decarboxylation of Δ^9-THC acid. The increase in Δ^8-THC content is caused by the simultaneous isomerization of Δ^9-THC originally present in the sample or formed by decarboxylation from Δ^9-THC acid. The decarboxylation of Δ^9-THC acid is indicated by the greater amount of Δ^8-THC after heating for 9 min than the amount of Δ^9-THC in the non-heated sample (the Δ^8-THC content after 9 min exceeds the Δ^9-THC content at 0 min).

The decarboxylation on silica sorbents modified with different salts was also studied and the results are summarized in Table II. Decarboxylation on silica modified with zinc acetate and ammonium acetate resulted in Δ^9-THC, whereas on silica modified with cobalt chloride, zinc, iron(II) and copper sulphates and ammonium bromide and chloride resulted in Δ^8-THC. On silica modified with ammonium oxalate, tartrate and perchlorate and aluminium fluoride both Δ^8- and Δ^9-THC were detected.

With sorbents modified with cobalt nitrate, copper acetate, ammonium thiocyanate and silver nitrate, none of the cannabinoids bound to the sorbent could be eluted, even using methanol.

Under the same conditions as given above, Δ^9-THC and CBD reference compounds were heated separately in a reactor tube packed with ammonium chloride-modified silica. The results verified the isomerization of Δ^9-THC. Conversion of CBD to THC also occurred. These results were also confirmed according to the previously mentioned methods.

On performing the decarboxylation on the sorbent surface the evaporation of neutral cannabinoids was hindered by sorptive effects, but chemical changes of the compounds formed also occurred, so the previous advantage of this method could not be used for quantification.

TABLE II

EFFECT OF DIFFERENT SALTS ON THE CHANGE IN THC AND CBN CONTENTS OF *n*-HEXANE MARIHUANA EXTRACT AFTER HEATING IN A SILICA-PACKED REACTOR TUBE AT 145°C FOR 10 MIN

Salt	Δ^9-THC (mg/g)	Δ^8-THC (mg/g)	CBN (mg/g)
Zn(II) acetate	4.74	0.00	4.78
NH_4 acetate	1.53	0.00	0.91
$CoCl_2$	0.00	5.00	3.87
$ZnSO_4$	0.00	5.41	4.65
$FeSO_4$	0.00	1.29	2.78
$CuSO_4$	0.00	4.29	4.35
NH_4Cl	0.00	7.29	5.56
NH_4Br	0.00	7.20	6.35
NH_4 oxalate	0.98	1.75	2.96
NH_4 tartrate	4.65	4.58	4.78
NH_4ClO_4	0.70	1.75	3.43
AlF_3	0.29	2.58	3.87

Decarboxylation in solvent phase

The decarboxylation of cannabinoid acids was studied in some high-boiling solvents such as ethylene glycol, diethylene glycol, *n*-octanol, dioctyl phthalate and dimethyl sulphoxide in an open reactor. In the temperature range 125–135°C the velocity of decarboxylation was low; at higher temperatures waste occurred owing to evaporation, which was confirmed by TLC. In these instances the mathematical correction could also be applied, which resulted in lower values of the velocity constant than for decarboxylation in an open reactor without solvent. The cannabinoid acid contents calculated from data obtained from decarboxylation at different temperatures showed the same results.

CONCLUSION

When performing the thermal decarboxylation of cannabinoid acids in either the presence or absence of organic solvents in an open reactor, an optimum temperature at which the velocity of the decarboxylation would be high enough and simultaneous evaporation of neutral cannabinoids would not occur could not be found. Consequently, it is not possible in this manner to obtain an amount of neutral cannabinoids equivalent to that of the cannabinoid acids from which they were decarboxylated. The systematic error caused by the time dependenc of the decarboxylation and the simultaneous evaporation in open reactors can be eliminated by the suggested mathematical correction.

During the decarboxylation on different sorbent surfaces, the evaporation of cannabinoids was hindered by sorptive effects, but simultaneous side-reactions occurred, causing chemical changes of the neutral cannabinoids.

A study on decarboxylation in closed reactors will be published later.

REFERENCES

1 E. Small and H. D. Beckstead, *Lloydia*, 136 (1973) 144.
2 R. N. Smith and C. G. Vaughan, *J. Chromatogr.*, 129 (1976) 347.
3 P. B. Baker, R. Fowler, K. R. Bagon and T. A. Gough, *J. Anal. Toxicol.*, 14 (1980) 145.
4 P. B. Baker, B. J. Taylor and T. A. Gough, *J. Pharm. Pharmacol.*, 33 (1981) 369.
5 R. Brenneisen, *Pharm. Acta Helv.*, 59 (1984) 247.
6 S. L. Kanter, M. R. Musumeci and L. E. Hollister, *J. Chromatogr.*, 171 (1979) 504.
7 R. N. Smith, *J. Chromatogr.*, 115 (1975) 101.
8 J. C. Turner and P. G. Mahlberg, *J. Chromatogr.*, 283 (1984) 165.
9 T. Veress, J. I. Szanto and L. Leisztner, *Magy. Kem. Foly.*, 95(2) (1989) 59.
10 M. Gurkin and J. Ripphahn, *Int. Lab.*, May/June (1980) 63.
11 P. Majlát (Editor), in *Proceedings of the Round Table Conference on Chemical Analysis of Narcotics and Dependence Causing Drugs, Budapest, October 5, 1973*, Hungarian Chemical Society, Budapest, 1974, p. 213.