Distinction of Synthetic Cannabidiol, Cannabichromene, and Cannabivarin by GLC Using On-Column Methylation

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Abstract □ The on-column flash methylation of synthetic cannabidiol, cannabichromene, and cannabivarin by trimethylanilinium hydroxide offers a rapid, simple GLC method for their distinction. The phenolic groups of other cannabinoids are methylated by the procedure described.

Keyphrases □ Cannabidiol, cannabichromene, and cannabivarin synthetic mixtures—GLC separation and analysis, on-column methylation □ Cannabichromene, cannabidiol, and cannabivarin synthetic mixtures—GLC separation and analysis, on-column methylation □ Cannabivarin, cannabidiol, and cannabichromene synthetic mixtures—GLC separation and analysis, on-column methylation □ Cannabinoid synthetic mixtures (cannabidiol, cannabichromene, and cannabivarin)—GLC separation and analysis □ GLC—separation and analysis, cannabidiol, cannabichromene, and cannabivarin synthetic mixtures, on-column methylation

Work in these laboratories has confirmed reports (1–3) that it is difficult to distinguish mixtures of synthetic cannabidiol (I), cannabichromene (II), and cannabivarin[1] (III) (4) by GLC analysis. Turner and Hadley (3) recently showed that trimethylsilylation of cannabidiol and cannabichromene gave the corresponding ethers, which were clearly separated by GLC on an OV-17 column, but cannabivarin was not examined in their study.

These three cannabinoids have phenolic groups which were expected to be amenable to on-column flash methylation using trimethylanilinium hydroxide (5, 6). The present study showed that the flash methylation technique offers a rapid and simple GLC method for the unequivocal distinction of the three pure compounds and for their separation in synthetic mixtures.

EXPERIMENTAL

GLC analyses were performed on two gas chromatographs[2], both equipped with flame-ionization detectors and fitted with 0.6-cm (0.25-in.) by 1.8-m (6-ft) glass columns packed with 5% OV-7[3] on 80–100-mesh Chromosorb W. Both the injection port and flame-ionization detector temperatures were 275°, and the nitrogen flow rate was 30 ml/min. Retention times were measured at several oven temperatures (Table I), and relative retention times were compared to the internal standard, 4-androstene-3,17-dione[4].

For methylation, 10 μl of a 0.5% cannabinoid solution in methanol (~0.16 μmole) was treated with 6–10 μl of 0.2 *M* trimethylanilinium hydroxide in methanol[5] (1.2–2 μmoles), and ~0.2 μl of the mixture was injected into the gas chromatograph. Emergent materials from the gas chromatograph was collected by extinguishing the detector flame and placing an open-ended glass capillary tube of the melting-point type over the jet at the predetermined time of peak emergence. The materials were examined by mass spectrometry[6] at an ionizing voltage of 70 ev and a probe temperature of 200°.

RESULTS AND DISCUSSION

Complete methylation of I, II, and III was shown to take place under the described conditions by the appearance of a single GLC peak and by examination of the emergent material by mass spectrometry. Thus, the mass spectrum of cannabidiol dimethyl ether had abundant ions at *m/e* (relative percent) 342 (14), 275 (36), 274 (100), 259 (11), 244 (21), 243 (91), 235 (19), 234 (17), 221 (67), 174 (13), 173 (57), and 158 (16). Cannabichromene methyl ether had abundant ions at *m/e* (relative percent) 328 (6), 313 (5), 246 (21), 245 (100), 231 (6), and 188 (8). Cannabivarin methyl ether had abundant ions at *m/e* (relative percent) 296 (6), 295 (21), 281 (35), 280 (100), 266 (9), 252 (9), 245 (9), 238 (21), and 209 (11).

Insufficient quantities of the trimethylanilinium hydroxide reagent resulted in the formation of the monomethyl ether of I, which had approximately the same retention time as the methyl ether of II. The mass spectrum of cannabidiol monomethyl ether had ions at *m/e* (relative percent) 328 (5), 327 (16), 313 (8), 286 (6),

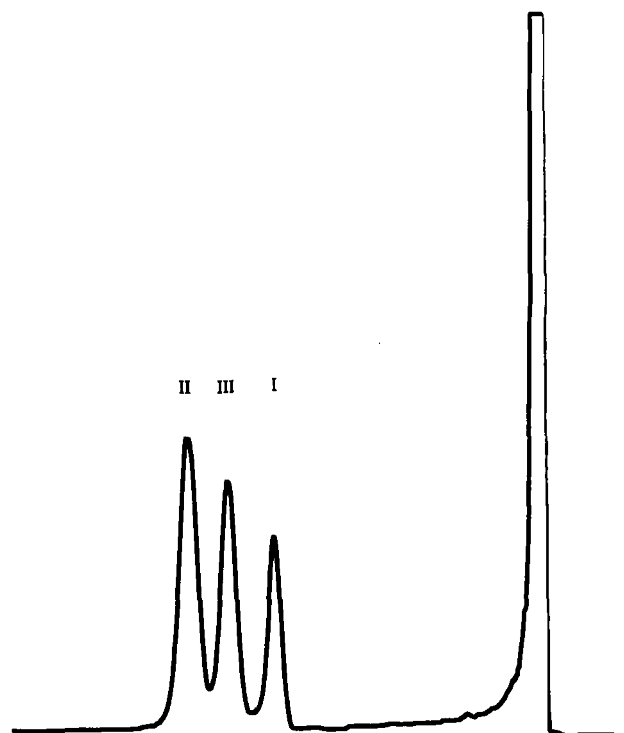


Figure 1—*Chromatogram obtained at 225° of a mixture of the dimethyl ether of cannabidiol (I) and the methyl ethers of cannabivarin (III) and cannabichromene (II).*

[1] Cannabivarin (the propyl homolog of cannabinol) and the other cannabinoids used in this study were prepared in these laboratories by published procedures; their identities were confirmed by proton magnetic resonance and mass spectral analyses.
[2] Varian Aerograph model A-700 and Hydro-Flow Systems Series 3000.
[3] A polar phenylmethyldimethylsilicone from Chromatographic Specialties, Ltd., Brockville, Ontario, Canada.
[4] See Ref. 3.
[5] Prepared and donated by Dr. K. K. Midha of these laboratories.
[6] Hitachi Perkin-Elmer RMU-6L.

Table I—GLC Retention Times and Relative Retention Times of Cannabinoids

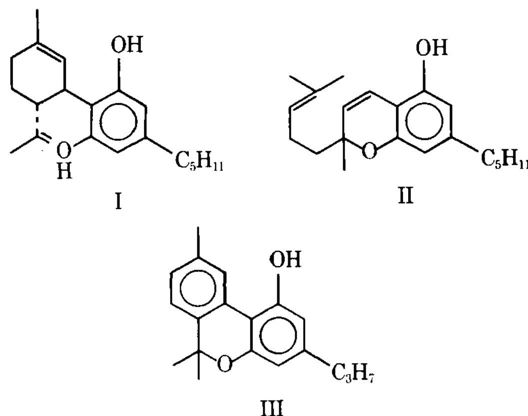
	Retention Time, min				Relative Retention Time	
	Column Temperature[a]		Column Temperature[b]		Column Temperature[b]	
Cannabinoid	225°	250°	190°	215°	190°	215°
Cannabivarin (III)	15.7	6.2	15.3	6.6	0.40	0.43
Cannabichromene (II)	15.7	6.2	16.6	7.0	0.44	0.45
Cannabidiol (I)	15.7	6.2	16.8	7.1	0.44	0.46
Δ^8-Tetrahydrocannabinol (IV)	19.8	7.8	21.0	8.5	0.55	0.55
Δ^9-Tetrahydrocannabinol (V)	21.5	8.2	22.8	9.2	0.60	0.59
Cannabigerol (VI)	24.9	9.3	26.2	10.0	0.69	0.65
Cannabinol (VII)	26.3	10.3	27.6	11.0	0.73	0.71
I dimethyl ether	8.5	3.8	9.3	4.3	0.24	0.28
III methyl ether	9.9	4.3	10.6	4.9	0.28	0.32
II methyl ether	11.1	4.6	11.7	5.2	0.31	0.34
I monomethyl ether	11.2	4.6	12.2	5.3	0.32	0.34
IV methyl ether	12.6	5.3	13.7	5.9	0.36	0.38
VI dimethyl ether	14.1	5.6	15.0	6.1	0.39	0.39
V methyl ether	14.4	5.8	15.6	6.4	0.41	0.41
VI monomethyl ether	16.8	6.9	18.0	7.6	0.47	0.49
VII methyl ether	17.6	7.1	19.1	7.8	0.50	0.50
4-Androstene-3,17-dione			38.0	15.5	1.00	1.00

[a] Varian Aerograph model A-700. [b] Hydro-Flow Systems Series 3000.

261 (10), 260 (41), 246 (27), 245 (100), 243 (9), 229 (8), 220 (9), and 207 (30).

Methylation of Δ^8-tetrahydrocannabinol (IV), Δ^9-tetrahydrocannabinol (V), cannabigerol (VI), and cannabinol (VII) also took place under these conditions; relative retention time and retention time data measured at two column temperatures on both instruments[2] are presented in Table I.

Synthetic cannabidiol, cannabichromene, and cannabivarin are easily distinguished by the simple methylation procedure described here. A chromatogram of a methylated mixture of the three is shown in Fig. 1. The applicability of the method to the determination of the cannabinoid content of *Cannabis* preparations, important when pharmacological studies are undertaken (7), remains to be investigated.



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