Constituents of *Cannabis sativa* L. III: Clear and Discrete Separation of Cannabidiol and Cannabichromene

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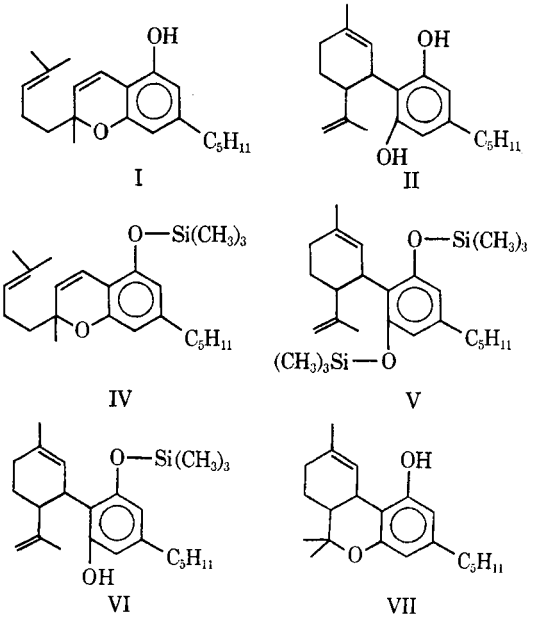
Abstract □ Synthetic cannabidiol and cannabichromene were discretely separated by GLC using their trimethylsilyl ether derivatives. The mono and disilylated derivatives of cannabidiol were identified. This procedure was utilized in the analysis of *Cannabis sativa* L.

Keyphrases □ *Cannabis sativa* L. constituents—separation of synthetic cannabidiol and cannabichromene using trimethylsilyl ether derivatives □ Cannabidiol—GLC separation from cannabichromene □ Cannabichromene—GLC separation from cannabidiol □ GLC—separation, cannabidiol and cannabichromene

Gaoni and Mechoulam (1) reported the separation of cannabichromene and cannabidiol by conventional GC techniques. However, this separation was based on retention times having only a 5-sec. time differentiation. With the identification of the propyl homologs by Vollner *et al.* (2), Gill *et al.* (3), and Merkus (4), there exists the possibility that, when used alone, GC analysis of cannabis constituents could lead to erroneous interpretations. Moreover, Vree *et al.* (5) showed that the propyl homolog of cannabinol, cannabivarin, was often found under the peak of a gas chromatogram normally attributed to cannabidiol or cannabichromene. These investigators were using hashish samples from Brazil and Lebanon; however, Fetterman and Turner (6) and Turner and Hadley (7) found propyl homologs in aerial parts of freshly harvested Indian *Cannabis sativa* L. grown in Mississippi. Additionally, they observed that cannabichromene was often reported as cannabidiol when GC was used as the only method for analysis (7). Accurate identification could, however, be obtained by using TLC and GC combined with a high-resolution mass spectrometer. The questioned validity of using only GC for the analysis of cannabidiol, cannabichromene, and cannabivarin prompted this investigation.



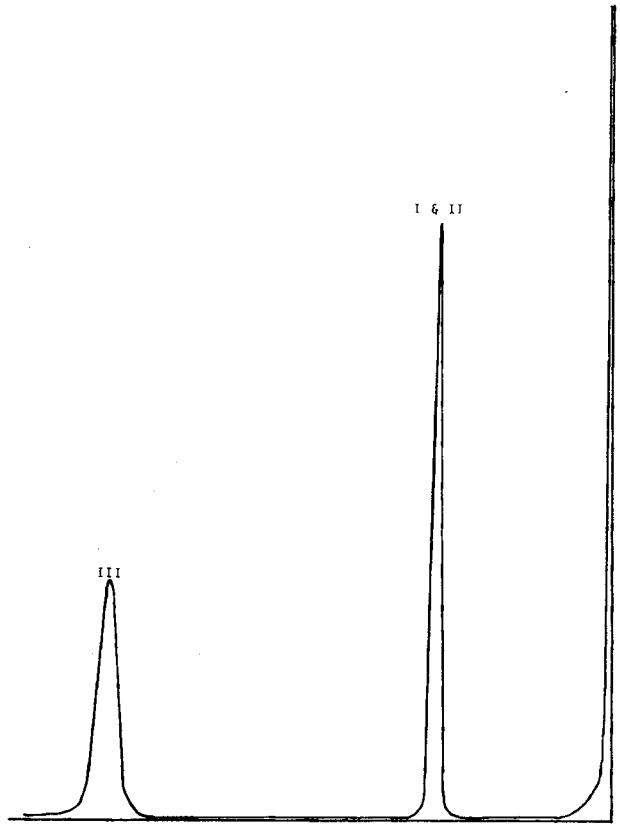


Figure 1—*Gas chromatogram of unsilylated cannabichromene (I), cannabidiol (II), and 4-androstene-3,17-dione, the internal standard (III).*

METHODS[1]

GC Analysis—Analyses were performed using gas chromatographs[2] equipped with hydrogen flame-ionization detectors and operated isothermally at 210°. The inlet temperature was 240° and the detector temperature was 260°. Glass columns [0.6-cm. (0.25-in.) o.d., 2-mm. i.d., × 2.4 m. (8 ft.)] were packed with 2% OV-17[3] on 100–120-mesh Chromosorb WHP. Nitrogen was used as the carrier gas at a flow rate of 10–16 ml./min., depending upon the instrument used.

[1] Authentic synthetic samples of cannabidiol and cannabichromene were obtained from the National Institute of Mental Health (NIMH). Relative retention times reported are relative to the internal standard 4-androstene-3,17-dione.

[2] Beckman GC-45 and GC 72-5.

[3] High purity polar phenyl methyl silicone of approximately 30,000 mol. wt. Packed by Beckman Instruments Co.

Table I—GC Relative Retention Times of Cannabinoids

Cannabinoid	Relative Retention Time
Cannabidiol bis(trimethylsilyl) ether	0.11
Cannabichromene trimethylsilyl ether	0.17
Cannabidiol mono(trimethylsilyl) ether	0.18
(−)-Δ^9-*trans*-Tetrahydrocannabinol trimethylsilyl ether	0.22
Cannabidiolic acid trimethylsilyl ester–bis(ether)	0.28
Cannabichromene	0.34
Cannabidiol	0.34
(−)-Δ^9-*trans*-Tetrahydrocannabinolic acid trimethylsilyl ester–ether	0.64
4-Androstene-3,17-dione	1.00

Silylation of Synthetic Cannabinoids—Five milligrams each of cannabidiol and cannabichromene was added to 0.2 ml. of anhydrous pyridine contained in a 50-ml. round-bottom single-neck flask. The pyridine solution was then subjected to continuous vibration from an ultrasonic vibrator until the cannabinoids were in solution. Then 0.3 mg. of the internal standard was added *via* a 10:1 pyridine–steroid solution. At this point, 0.4 ml. of *N,O*-bis(trimethylsilyl)-trifluoroacetamide with 1% trimethylchlorosilane[4] was added. The resulting reaction mixture was heated, using a heating mantle, for approximately 10 min. at 80°. Then 0.1 μl. of the reaction mixture was injected into the gas chromatograph.

Silylation of Plant Extract—A 1-g. sample was extracted with 40 ml. of spectrograde chloroform. The resulting solution was refrigerated at 6° and shaken at 10-min. intervals for 1 hr. The plant material then was removed by filtration, and the mother liquor was concentrated *in vacuo* at ambient temperature to a greenish paste void of solvent.

Anhydrous pyridine, 0.5 ml., was added, followed by continuous vibration from an ultrasonic vibrator until all resin was in solution. At this point, 0.5 ml. of *N,O*-bis(trimethylsilyl)-trifluoroacetamide[4] with 1% trimethylchlorosilane was added. The resulting reaction mixture was then processed as described previously for the silylation of synthetic cannabinoids. Unsilylated plant samples of *C. sativa* L. were analyzed according to the literature procedure (7).

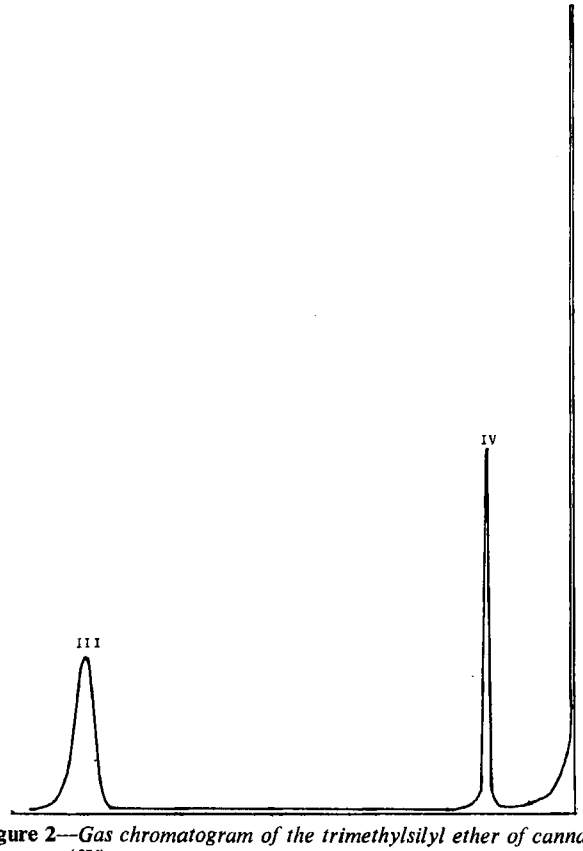


Figure 2—*Gas chromatogram of the trimethylsilyl ether of cannabichromene (IV).*

[4] BSTFA with 1% TMCS, Pierce Chemical Co.

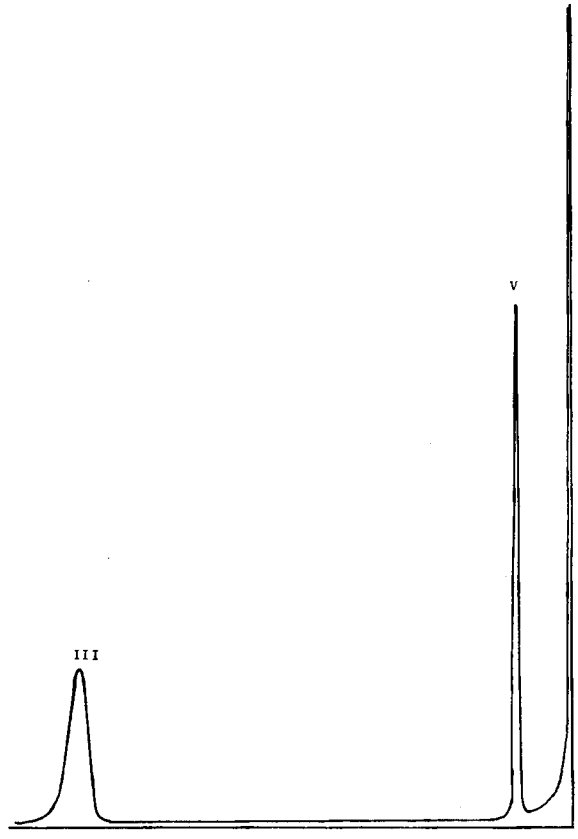


Figure 3—*Gas chromatogram of bis(trimethylsilyl) ether of cannabidiol (V).*

RESULTS AND DISCUSSION

The structures of natural and synthetic cannabichromene (I) and cannabidiol (II) are well known (1). Individual analysis of each of these cannabinoids is carried out routinely; however, when a sample contains both cannabidiol and cannabichromene (Fig. 1), no clean separation is obtained. This presents many problems to the researcher trying to ascertain if the sample contains a mixture of the two or is a pure sample of either. Claussen *et al.* (8) used the trimethylsilyl ether–ester derivatives to separate the free phenols from the carboxylic acid derivatives of some cannabinoids. Fetterman *et al.* (9) used a similar procedure to analyze for (−)-Δ^9-*trans*-tetrahydrocannabinolic acid. Claussen *et al.* (8) were unable to identify many peaks in the chromatogram obtained, whereas Fetterman *et al.* (9) used fresh marijuana having a very high concentration of (−)-Δ^9-*trans*-tetrahydrocannabinolic acid (approximately 90–97%) and a very small content of (−)-Δ^9-*trans*-tetrahydrocannabinol. Additionally, Davis *et al.* (10) described a silylated extract of cannabis. These three procedures are excellent but do not approach the problem of separating cannabidiol and cannabichromene as their free phenols.

A clean separation has been obtained by using the trimethylsilyl ethers of synthetic and natural cannabichromene and cannabidiol. Figure 1 is a chromatogram of equal parts (2 mg.) of synthetic cannabichromene and cannabidiol, analyzed as described under the *GC Analysis* section. The relative retention time of both compounds when compared to the internal standard 4-androstene-3,17-dione (III) is 0.34. Figure 2 is a chromatogram of the trimethylsilyl ether of synthetic cannabichromene (IV) having a relative retention time of 0.17, and Fig. 3 is a chromatogram of the bis(trimethylsilyl) ether of cannabidiol (V) having a relative retention time of 0.11.

Several factors affect the efficiency of silylation. The most common is incorrect handling of the sample. No solvents having an

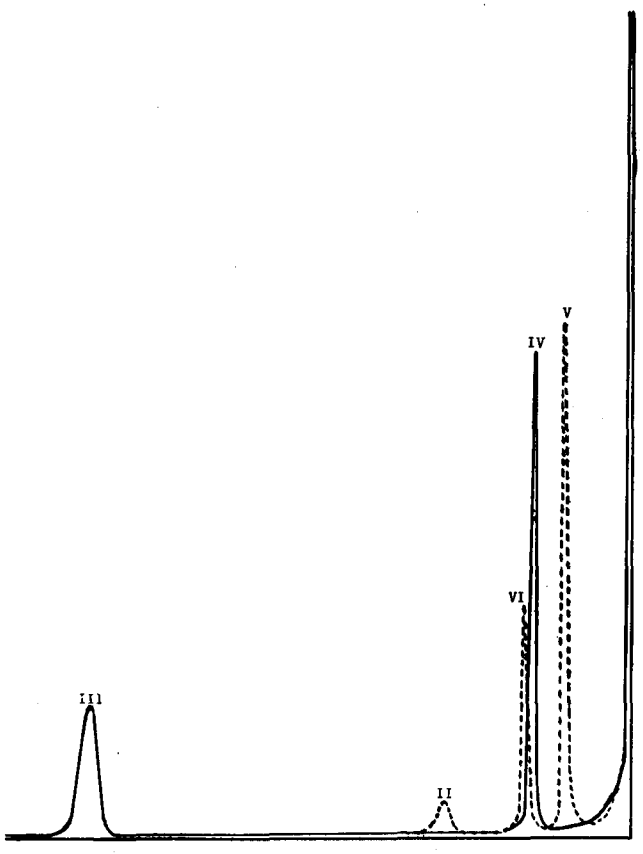


Figure 4—*Overlay of incompletely silylated cannabidiol and silylated cannabichromene, showing cannabidiol (II), mono(trimethylsilyl) ether of cannabidiol (VI), trimethylsilyl ether of cannabichromene (IV), and bis(trimethylsilyl) ether of cannabidiol (V). Key: ——, silylated synthetic cannabichromene; and - - -, products of incompletely silylated synthetic cannabidiol.*

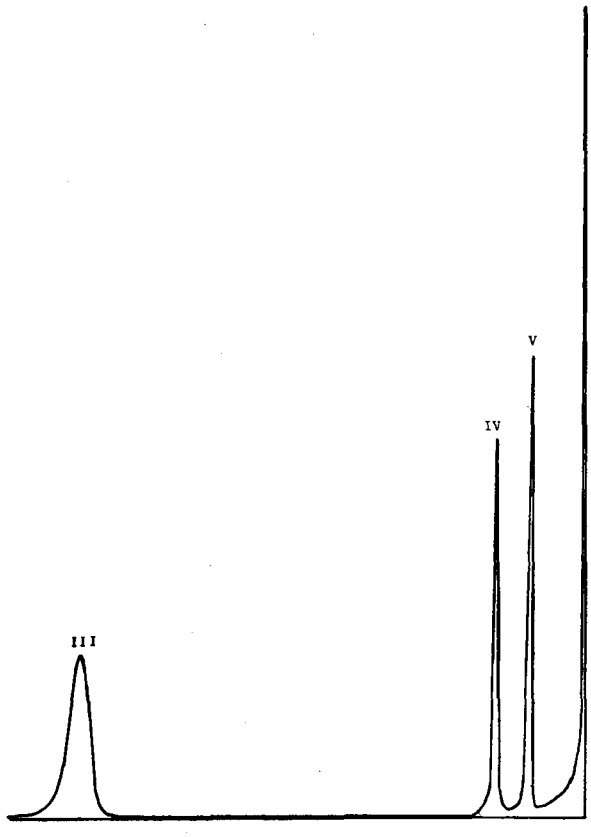
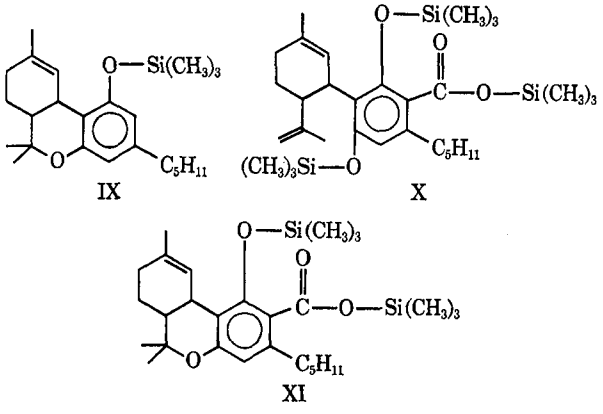


Figure 5—*Chromatogram of the trimethylsilyl ether of cannabichromene (IV) and the bis(trimethylsilyl) ether of cannabidiol (V).*

easily ionizable proton should be used. The silylation should be carried out with freshly obtained anhydrous solvents or solvents stored over drying agents such as molecular sieves.

Incomplete silylation can also be caused by an insufficient reaction time, too weak a silylating reagent, and/or an insufficient amount of silylating solution. For best results, an excess of the silylating solution should be added. Figure 4 is an overlay of an insufficient amount of a silylating solution being added to an equal weight of cannabidiol and cannabichromene. The solid line represents the trimethylsilyl ether of cannabichromene (IV), and the broken line represents silylated products of cannabidiol and free cannabidiol. The peak labeled II is unsilylated cannabidiol. The peak labeled VI is the mono(trimethylsilyl) ether of cannabidiol, and the peak labeled V is the bis(trimethylsilyl) ether of cannabidiol.

With the addition of a sufficient amount of the silylating solution, II and VI disappear. Synthetic cannabichromene and cannabidiol



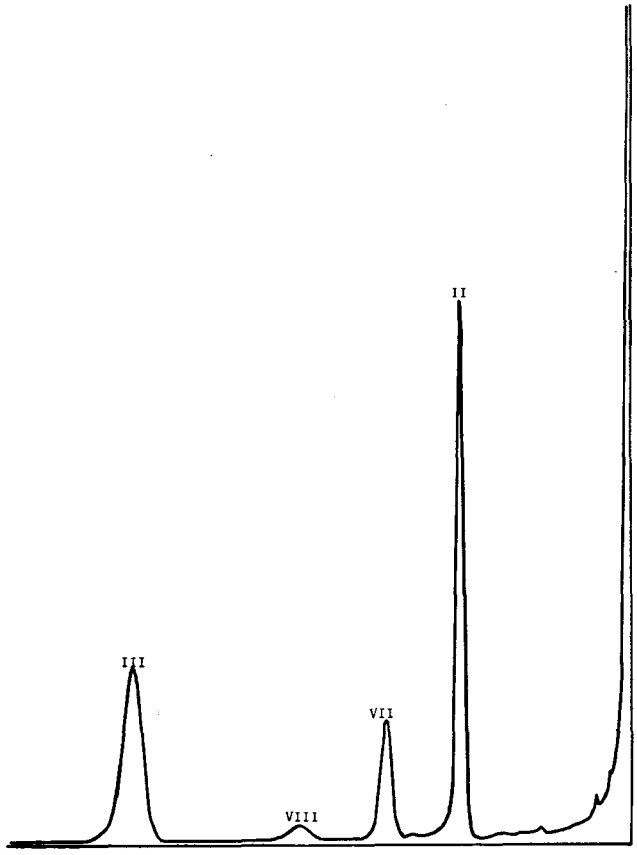


Figure 6—*Chromatogram of unsilylated Turkish (TU-A) C. sativa L., showing cannabidiol (II), (—)-Δ⁹-trans-tetrahydrocannabinol (VII), and unknown constituent (VIII).*

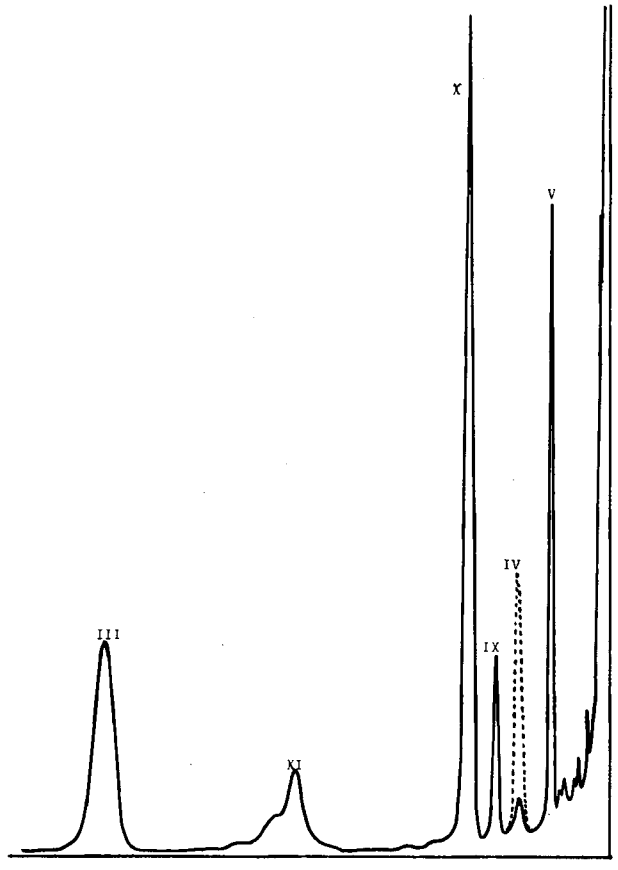


Figure 7—*Overlay of silylated synthetic cannabichromene and Turkish (TU-A) C. sativa L., showing bis(trimethylsilyl) ether of cannabidiol (V), trimethylsilyl ether of cannabichromene (IV), trimethylsilyl ether of (—)-Δ⁹-trans-tetrahydrocannabinol (IX), trimethylsilyl ester–bis(ether) of cannabidiolic acid (X), and trimethylsilyl ester–ether of (—)-Δ⁹-trans-tetrahydrocannabinolic acid (XI). Key: ——, silylated Turkish variant; and - - -, silylated Turkish variant plus synthetic cannabichromene.*

can be discretely separated when a slight excess of silylating solution is used (Fig. 5). Care must be taken, however, to ensure complete silylation since the mono(trimethylsilyl) ether of cannabidiol (Fig. 4) has a relative retention time of 0.18, very near that of the cannabichromene trimethylsilyl ether at 0.17 (Table I).

To illustrate the versatility and application of this procedure, a sample of Turkish *C. sativa* L. was analyzed (Fig. 6). Regular cannabis analysis decarboxylates cannabinoid acids to their free phenol. Thus, cannabidiol (II) and (−)-Δ⁹-*trans*-tetrahydrocannabinol (VII), along with an unknown constituent (VIII), are shown in Fig. 6. The unknown constituent is under further investigation at this time. Without close observation, VIII would normally be called cannabinol. However, cannabinol has a relative retention time of 0.63, whereas VIII is located at 0.67. Both cannabinol and VIII occur in some plant samples, particularly Mexican. Usually VIII appears as a shoulder immediately after cannabinol.

Silylated cannabinoids from a Turkish variant of *C. sativa* L. are shown in Fig. 7. The bis(trimethylsilyl) ether of cannabidiol is V. Peak IV is the trimethylsilyl ether of cannabichromene. The broken line represents a cannabichromene-enriched plant sample. Peak IX is the trimethylsilyl ether of (−)-Δ⁹-*trans*-tetrahydrocannabinol, and X is the trimethylsilyl ester–bis(ether) of cannabidiolic acid. The trimethylsilyl ester–ether of (−)-Δ⁹-*trans*-tetrahydrocannabinolic acid (XI) is followed by a shoulder of an unknown component.

Thus, trimethylsilyl derivatives of cannabinoids can be used to differentiate if a plant sample of *C. sativa* L. contains cannabidiol, cannabichromene, or a mixture of the two. However, caution must be observed to ensure complete silylation, and/or the absence of the internal standard will lead to erroneous results.

SUMMARY

Synthetic cannabidiol and cannabichromene are separated as their trimethylsilyl ethers. The mono(trimethlysilyl) ether of cannabidiol is identified. The procedure described is applicable to samples of *C. sativa* L. plant material when the defined conditions are observed. Therefore, it is possible to determine qualitatively, using GC, if a sample of cannabis contains cannabichromene, cannabidiol, or a mixture of the two.

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