Journal of Chromatography, 237 (1982) 389–397
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,492

GAS CHROMATOGRAPHY OF CANNABIS CONSTITUENTS, INCLUDING CANNABINOID ACIDS, WITH ON-COLUMN ALKYLATION AND ESTERIFICATION

SVEN BJÖRKMAN*
Psychiatric Research Centre, Ulleråker Hospital, S-750 17 Uppsala (Sweden)
(Received October 15th, 1981)

SUMMARY

Neutral cannabis constituents and cannabinoid acids were gas chromatographed with on-column methylation of phenolic hydroxyl groups and esterification of carboxylic acid functions. Injection of cannabis extracts as solutions in 2 *M* dimethylformamide dimethylacetal in pyridine gave quantitative or near-quantitative conversion of cannabinol and tetrahydrocannabinol into methyl ethers, while cannabidiol gave a monomethyl and a dimethyl derivative. Also cannabidiolic acid, tetrahydrocannabinolic acid and the tetrahydrocannabinol 7-acid metabolite chromatographed well as methyl esters and ethers. The mass spectra of the methyl derivatives gave more pertinent structural information than those of the corresponding TMS derivatives. This simple derivatization procedure should conceivably prove useful for the analysis of other heat-labile biological samples.

INTRODUCTION

Gas chromatography (GC) of neutral cannabis constituents is a simple procedure[1]. Tetrahydrocannabinol (THC), cannabidiol (CBD) and cannabinol (CBN) with their propyl and methyl homologues elute as symmetric peaks from packed[2] as well as capillary columns[3]. Cannabinoid acids[1,4], on the other hand, cannot be gas chromatographed as such. If, for instance, Δ^1-tetrahydrocannabinol-4′-carboxylic acid is introduced into the GC injector it will decarboxylate and give THC[5,6]. The cannabinoid acids are consequently derivatized prior to analysis and chromatographed as trimethylsilyl (TMS) derivatives, with TMS protection of phenolic as well as carboxylic acid functions[5–9], or as methyl esters with TMS protection of phenolic functions only[9–11]. In the preparation of TMS derivatives, however, the reaction mixture must be heated, which may cause decomposition of some cannabinoid constituents[9]. Also, the mass spectra of the TMS derivatives sometimes yield very little structural information[9,12].

Dimethylformamide (DMF) dialkylacetals are excellent derivatizing agents for

* Present address: Department of Microbiology (N), Biomedical Center, S-751 23 Uppsala, Sweden

0021-9673/82/0000–0000/$02.75

carboxylic acids[13]. The reaction mixture of, for instance, DMF dimethylacetal and a fatty acid in pyridine is usually injected directly onto the GC column, without prior heating, to give a quantitative yield of fatty acid methyl ester. The DMF dialkylacetals also alkylate phenolic hydroxyl functions[14]. They are consequently potential derivatizing agents for neutral as well as acidic cannabis constituents, and this paper presents results demonstrating their practical usefulness. DMF dimethylacetal was used to alkylate cannabinoids under the mildest possible conditions, and the mass spectra of the derivatives proved readily interpretable.

EXPERIMENTAL

Samples and reagents

Samples (100 mg) of cannabis resin (hashish) or marihuana, submitted for forensic analysis, were triturated with 1 ml of methanol[15]. The suspension was placed in an ultrasonic bath for 20 min and then centrifuged. Aliquots of the clear supernatant were either injected on to the GC column or derivatized as described below. Sample extraction, derivatization and analysis were performed on the same day, and the solutions were kept in the dark when not in use.

Synthetic Δ^1-tetrahydrocannabinol-7-acid (NIH, Bethesda, U.S.A.) was a gift from Drs. Magnus Halldin and Stig Agurell. Dimethylformamide dimethylacetal, 2 *M* in pyridine, Methyl-8®, was purchased from Pierce (Rockford, IL, U.S.A.), as was the GC column packing, OV-1, 3% on Chromosorb W HP, 80–100 mesh.

Derivatization procedure

Aliquots (25 μl) of the methanolic cannabis extracts were transferred to conical, glass-stoppered test-tubes, and the solvent was evaporated under a nitrogen stream. The residues were then dissolved in Methyl-8 (100 μl). Samples (2–4 μl) of these solutions were injected on to the GC column. In addition, a "sandwich" injection was tried; 1 μl of hashish extract followed by 5 μl of reagent in the same syringe.

Δ^1-Tetrahydrocannabinol-7-acid was injected as a freshly prepared 10^{-3} *M* solution in Methyl-8.

Equipment and gas chromatographic conditions

The combined gas chromatograph–mass spectrometer was a Finnegan 4023 EI/CI system interfaced with an INCOS data system (Sunnyvale, CA, U.S.A.).

The gas chromatograms were run on a 196 cm × 2 mm I.D. glass column packed with 3% OV-1. The helium flow-rate was 25 ml/min, the injector block temperature was 300°C and the glass jet separator was kept at 250°C. The derivatized or underivatized cannabis extracts were analysed in temperature-programmed runs: the oven temperature was first maintained for 2 min at 200°C, then raised by 10°C/min to 260°C and finally kept there for 5 min. The derivative of Δ^1-THC-7-acid was also analysed at 270°C oven temperature.

Mass spectra were recorded continuously, at one scan every 3 sec, and stored on the data disc. The ion source temperature was 250°C, the electron energy was 70 or 23 eV, the emission current was 0.28 mA, the EM voltage was −1960 V and the preamplifier sensitivity was 10^{-7} A/V. When chemical ionization (CI) spectra were recorded, isobutane (99.5% pure, AGA, Lidingö, Sweden) was added as make-up gas

to an indicated ion source pressure of 27 pA (0.20 torr). The ionizing current was 0.25 mA at 70 eV, the EM voltage was −1850 V and the preamplifier sensitivity was 10^{-8} A/V.

The data-processing included the plotting of chromatograms as reconstructed ion currents (RICs) and of background-subtracted mass spectra. The data system also allows the construction of mass chromatograms, ion intensity *vs.* time, at any *m/z* value. The mass chromatograms can be used to distinguish the mass spectra of two or more substances eluting as an unresolved GC peak and to check whether a peak in a spectrum belongs to "sample" or to "background". They can also, as in selected ion monitoring, be used for quantitation as well as for the localization of compounds with known mass spectra, that elute in amounts too small to give noticeable peaks in the RIC chromatograms.

RESULTS AND DISCUSSION

Gas chromatograms of underivatized and derivatized cannabis extracts

Figs. 1 and 2 show the gas chromatograms of cannabis extracts with and without on-column derivatization. Tables I and II list the compounds identified in the four GC–MS runs, and their structures are given in Fig. 3. The small peaks of underivatized THC (their origin is discussed below) in the chromatograms B and D serve as reference peaks for the comparison of retention times between the four chromatograms.

The methyl ether derivatives of tetrahydrocannabivarin, THC and CBN elute 42, 51 and 51 sec earlier, respectively, than the underivatized compounds. In the

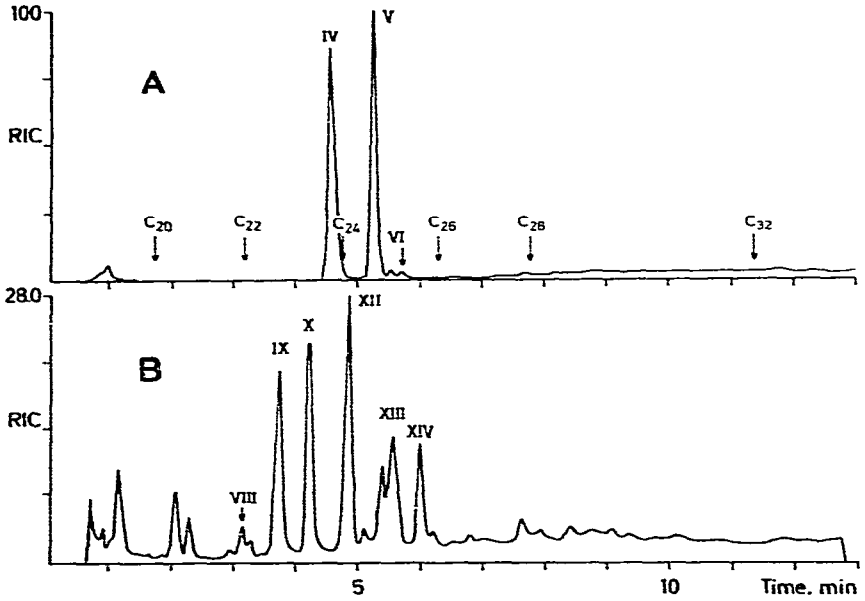

Fig. 1. Temperature-programmed GC–MS runs (reconstructed ion current chromatograms) of (A) a marihuana extract and (B) the same extract with on-column methylation. The arrows, marked C_{20} etc., indicate the positions of the peaks from a reference mixture of straight-chain hydrocarbons run under the same conditions. The roman numerals refer to Tables I and II and to Fig. 3.

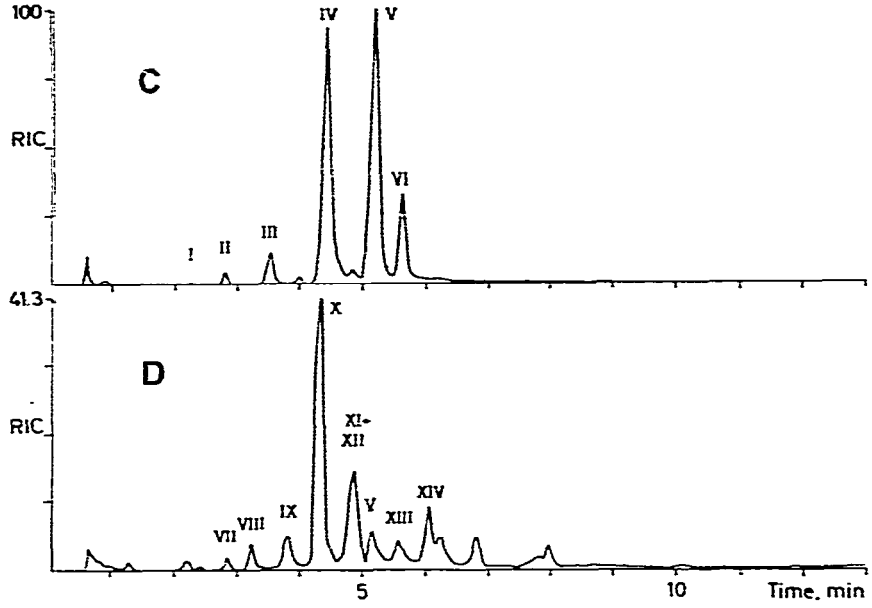

Fig. 2. Temperature-programmed GC–MS runs (reconstructed ion current chromatograms) of (C) a hashish extract and (D) the same extract with on-column methylation. The roman numerals refer to Tables I and II and to Fig. 3.

temperature programme 42 sec corresponds to an elution temperature 7°C lower and 51 sec to an elution temperature 8.5°C lower. Cannabidiol appears as two derivatives, a monomethyl ether (IX) eluting 36 sec (6°C) earlier than the underivatized compound and a dimethyl ether (VIII) eluting another 36 sec (6°C) earlier. The chromatograms B and D contain at least five distinct peaks due to methyl esters of carboxylic

TABLE I

IDENTIFIED CANNABIS CONSTITUENTS IN CHROMATOGRAMS A AND C

Retention time (min) in chromatogram		*Compound*	*Molecular weight**
A	*C*		
–	2.30	Tetrahydrocannabiorcol (I)	258
–	2.80	Cannabidivarin (II)	286
–	3.55	Tetrahydrocannabivarin (III)	286
4.55	4.45	Cannabidiol (IV)	314
5.25	5.20	Tetrahydrocannabinol (V)	314
5.55	–	(Dioctylphthalate)**	
5.70	5.65	Cannabinol (VI)	310

* The sample was re-run under identical GC conditions but with 23 eV electron energy, which afforded clear identification of all molecular ions.

** A ubiquitous contaminant from plasticizers.

TABLE II

IDENTIFIED COMPOUNDS IN CHROMATOGRAMS B AND D

Retention time (min) in		*Compound*	*Molecular weight**
B	*D*		
1.15	1.30	Methyl palmitate	270
2.05	2.20	(Unidentified)	296
2.35	2.40	Methyl stearate	298
–	2.85	3'-O-Methyltetrahydrocannabivarin (VII)	300
3.15	3.25	1'-O, 3'-O-Dimethylcannabidiol (VIII)	342
3.75	3.85	O-Methylcannabidiol (IX)**	328
4.25	4.35	3'-O-Methyltetrahydrocannabinol (X)	328
–	4.80	3'-O-Methylcannabinol (XI)	324
4.85	4.90	1'-O, 3'-O-Dimethylcannabidiolic acid methyl ester (XII)	400
5.10	5.20	Tetrahydrocannabinol (V)	314
5.40	–	(Dioctylphtalate)***	
5.55	5.60	O-Methylcannabidiolic acid methyl ester (XIII)§	386
6.00	6.10	3'-O-Methyltetrahydrocannabinolic acid methyl ester (XIV)	386
6.20	6.30	(Unidentified)	438
–	6.85	(Unidentified)	441
–	8.00	(Unidentified)	381

* The samples were re-run under identical GC conditions but with the mass spectrometer operating in the CI mode. The isobutane CI spectra afforded unambiguous determination of all molecular weights. Indeed, the CI spectra of compounds VII, X, XI and V consisted of an intense $[M+H]^+$ ion peak and no fragment peaks of more than 5% abundance.

** The two phenolic functions of cannabidiol are equivalent.

*** Contaminant from plasticizers.

§ The two phenolic functions of cannabidiolic acid are not equivalent, and the peak is probably due to a mixture of the two isomers.

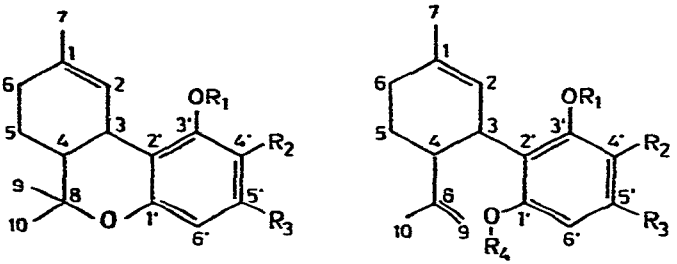

Fig. 3. The structures of the cannabis constituents and their derivatives. The following compounds are represented by formula A: I, $R_1=R_2=H$, $R_3=CH_3$; III, $R_1=R_2=H$, $R_3=C_3H_7$; V, $R_1=R_2=H$, $R_3=C_5H_{11}$; VI, as V, but the C^{1-6} ring aromatic; VII, $R_1=CH_3$, $R_2=H$, $R_3=C_3H_7$; X, $R_1=CH_3$, $R_2=H$, $R_3=C_5H_{11}$; XI, As X, but the C^{1-6} ring aromatic; XIV, $R_1=CH_3$, $R_2=COOCH_3$, $R_3=C_5H_{11}$. Formula B represents: II, $R_1=R_2=R_4=H$, $R_3=C_3H_7$; IV, $R_1=R_2=R_4=H$, $R_3=C_5H_{11}$; VIII, $R_1=CH_3$, $R_2=H$, $R_3=C_5H_{11}$, $R_4=CH_3$; IX, $R_1=R_2=H$, $R_3=C_5H_{11}$, $R_4=CH_3$; XII, $R_1=CH_3$, $R_2=COOCH_3$, $R_3=C_5H_{11}$, $R_4=CH_3$; XIII, $R_1=CH_3$, $R_4=H$ (or vice versa), $R_2=COOCH_3$, $R_3=C_5H_{11}$.

acids. The di- and monomethyl ether derivatives of cannabidiolic acid methyl ester (XII and XIII) and 3'-O-methyltetrahydrocannabinolic acid methyl ester (XIV) elute in the same order and with the same time intervals as the corresponding neutral cannabinoid derivatives. The increase in elution time due to the added methoxycarbonyl functions is 103 ± 2 sec for the three compounds, which corresponds to an elution temperature 17°C higher.

Derivatization yields

The on-column derivatization of cannabinol was more than 99.5% complete, as estimated from the mass chromatograms at $m/z = 309$ (base peak of the derivative, see Table III) and $m/z = 295$ (base peak of CBN). The proportion of underivatized to derivatized THC was estimated by quantitation of the mass chromatogram peaks given by the $m/z = 313$ and $m/z = 299$ ions. This procedure was regarded as permissible, even though the relative molar responses of the two compounds may be slightly different, because the mass spectral behaviour of THC changes very little on methylation (*cf.* Table III). The proportion of THC to methylated THC was 6.1 ± 2.7% (mean ± S.D. of eleven experiments) under the conditions described. When the evaporation residue of 10 µl of cannabis extract was treated with 200 µl of reagent, no underivatized THC could be detected in the gas chromatogram, but the rather high dilution of the sample is a disadvantage in the GC–MS analysis. However, because underivatized tetrahydrocannabinolic acid decarboxylates and also appears as THC in the chromatogram, this peak represents the total amount of underivatized material from two components of the sample, and the methylation yield can stand comparison with that of silylation, which is typically 95–97%[8]. Cannabidiol, as already described, gives two derivatives. Judging by the mass chromatograms at $m/z = 231$ (the base peak of CBD) there is no underivatized cannabidiol left.

The "sandwich" injection of cannabis extract and reagent afforded 76% methylation of cannabinol and 37% methylation of tetrahydrocannabinol (one experiment only).

TABLE III

THE MASS SPECTRA OF METHYLATED NEUTRAL CANNABINOIDS COMPARED WITH THOSE OF THEIR PARENT COMPOUNDS

The major peaks (above $m/z = 100$) of the 70 eV spectra are presented.

Tetrahydrocannabivarin (III)	203(100),	243(75),	–,	271(93),	286(85)		
O-Methyl derivative (VII)	217(60),	257(59),	269(19),	285(100),	300(86)		
Tetrahydrocannabinol (V)	231(100),	243(43),	258(30),	271(57),	–,	299(91),	314(83)
O-Methyl derivative (X)	245(59),	257(37),	272(12),	285(38),	297(21),	313(100),	328(84)
Cannabidiol (IV)	121(17),	–,	–,	174(17),	193(13),	–,	–,
O-Methyl derivative (IX)	–,	–,	174(8),	188(8),	–,	–,	–,
Dimethyl derivative (VIII)	–,	173(35),	–,	–,	221(65),	235(14),	243(44),
	231(100),	246(18),	299(2),	314(7)			
	245(100),	–,	313(3),	328(1)			
	–,	274(100),	–,	342(2)			
Cannabinol (VI)	193(29),	–,	–,	238(19),	295(100),	310(12)	
O-Methyl derivative (XI)	–,	209(12),	238(12),	252(9),	309(100),	324(14)	

The mass spectra of the derivatives

The mass spectra of the derivatives are given in Tables III and IV.

TABLE IV

THE MASS SPECTRA OF THE CANNABINOID ACID DERIVATIVES

The major peaks (above $m/z = 100$) of the 23 eV spectra are presented.

1'-O, 3'-O-Dimethylcannabidiolic acid methyl ester (XII)	249(14), 263(32), 267(25), 269(18), 279(100), 294(28), 332(99), 353(10), 368(13), 369(13), 400(20)
O-Methylcannabidiolic acid methyl ester (XIII)*	245(1), 303(100), 371(2), 386(8)
3'-O-Methyltetrahydrocannabinolic acid methyl ester (XIV)	270(2), 303(2), 315(3), 330(4), 339(20), 354(8), 355(8), 371(12), 386(100)

* Table II, note [a].

The well-explored[16–19] fragmentation paths of tetrahydrocannabinol and tetrahydrocannabivarin are little affected by the introduction of 3'-O-methyl groups. The only major peaks in the spectra of derivatives VII and X that do not have exact counterparts (14 mass units lower) in those of the unmethylated compounds are the ones at M−31 (loss of CH_3O). Cannabidiol, as well as THC, undergoes fragmentation by two competing paths: loss of carbon atoms 4, 5 and 8–10 in a retro-Diels–Alder reaction gives $m/z = 246$, and loss of carbon atoms 4–6 and 8–10, with ring-closure between carbon 1 and a phenolic oxygen, gives a chroman-like fragment at $m/z = 231$[16,18–20]. The latter fragmentation takes place in the monomethyl derivative IX, giving $m/z = 245$, but it is blocked when both phenolic groups are methylated. Instead, for compound VIII the retro-Diels–Alder fragmentation predominates, giving $m/z = 274$ and, with further loss of carbon atoms 1, 2, 6 and 7, $m/z = 221$ (a tropylium ion)[20]. Cannabinol fragments by the loss of a methyl group to give the base peak at $m/z = 295$ and by further loss of a butyl radical from the side-chain giving $m/z = 238$[16]. This holds true also for the methyl ether XI, but the spectrum of this compound still has a peak at $m/z = 238$, possibly indicating a loss of the whole side-chain.

The mass spectrum of cannabidiolic acid methyl ester dimethyl ether (XII) is rather complicated. The expected clevage of CH_3O from the methyl ester function can, however, be observed ($m/z = 369$, which is the base peak of the isobutane CI spectrum) as well as the loss of carbon atoms 4, 5, 8, 9 and 10 in a retro-Diels–Alder reaction ($m/z = 332$) and the further loss of carbon atoms 1, 2, 6 and 7, which gives a tropylium type ion at $m/z = 279$; *cf.* the fragmentation of compound VIII (Table III) and of cannabidiolic acid methyl ester[21]. The fragmentation of the monomethyl ether (XIII) occurs predominantly by loss of carbon atoms 4–6 and 8–10 with ring-closure to a chroman-like fragment ($m/z = 303$; *cf.* the spectra of CBD and compound IX). There is also a small M−15 peak. The same fragmentations can be discerned in the spectrum of the tetrahydrocannabinolic acid derivative XIV, which, however, has a much more intense molecular ion peak; *cf.* the difference between the spectra of the analogous neutral cannabinoid derivatives IX and X. This compound also loses

CH_3O and CH_3OH (m/z = 355 and 354) and gives a fragment at m/z = 339, which probably implies a loss of CH_3OH and a methyl group. There is a corresponding peak at m/z = 325 in the mass spectrum of Δ^6-tetrahydrocannabinolic acid methyl ester[22].

Derivatization of tetrahydrocannabinol-7-acid

Δ^1-THC-7-acid, a major metabolite of THC in man[23], gives a derivative with a very simple mass spectrum (Fig. 4). The molecular weight is 372, m/z = 357 equals a loss of a methyl group and m/z = 313 loss of the whole methoxycarbonyl function. This acid is a more polar compound than the naturally occurring cannabinoid acids, and its derivative elutes rather late in the temperature-programmed GC runs, at 7 min. At 270°C column temperature, the retention time of the derivative is 1.2 min.

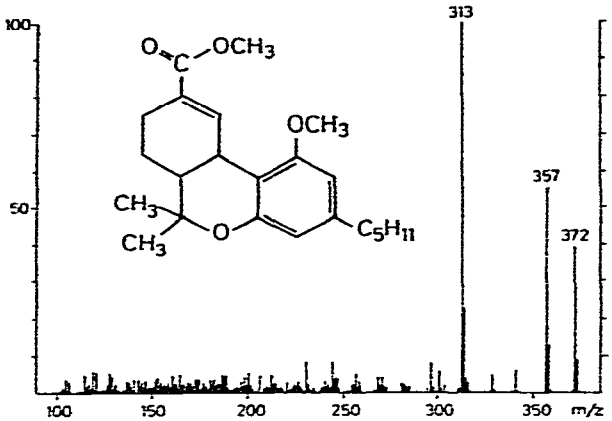

Fig. 4. The structure and 70-eV mass spectrum of 3'-O-methyltetrahydrocannabinol-7-acid methyl ester.

CONCLUSIONS

The usefulness of on-column alkylation and esterification of heat-labile phenols and phenolic acids has been evaluated with cannabis constituents as model compounds. The methylation of the monophenolic compounds CBN and THC was quantitative or near-quantitative, while the diphenolic CBD appeared as two derivatives in the chromatograms. The derivatization yields of the acid cannabinoids could not be estimated owing to lack of reference substances. The carboxylic acid functions can, however, be assumed to react faster than the phenolic ones[14]. The mass spectra of the derivatives are generally readily interpretable and should compare favourably with those of the corresponding TMS derivatives, which are often dominated by an intense M − 15 peak due to cleavage of a methyl group from the TMS function itself[9,12]. This very simple and rapid derivatization method should conceivably prove useful in the qualitative or quantitative analysis of other complex mixtures, such as plant extracts or other biological samples.

ACKNOWLEDGEMENT

This work was performed as part of a drug analysis programme supported by The Delegation for Social Research (project number 78/53).

REFERENCES

1 R. Mechoulam, N. K. McCallum and S. Burstein, *Chem. Rev.*, 76 (1976) 75.
2 T. B. Vree, D. D. Breimer, C. A. M. Van Ginneken and J. M. Van Rossum, *J. Chromatogr.*, 74 (1972) 209.
3 M. Novotny, M. L. Lee, C.-E. Low and A. Raymond, *Anal. Chem.*, 48 (1976) 24.
4 C. E. Turner, M. A. Elsohly and E. G. Boeren, *J. Natural Prod.*, 43 (1980) 169.
5 K. H. Davis, N. H. Martin, C. G. Pitt, J. W. Wildes and M. E. Wall, *Lloydia*, 33 (1970) 453.
6 P. S. Fetterman, N. J. Doorenbos, E. S. Keith and M. W. Quimby, *Experientia*, 27 (1971) 988.
7 C. E. Turner and K. W. Hadley, *J. Pharm. Sci.*, 62 (1973) 1083.
8 C. E. Turner, K. W. Hadley, J. Henry and M. L. Mole, *J. Pharm. Sci.*, 63 (1974) 1872.
9 S. Billets, F. El-Feraly, P. S. Fetterman and C. E. Turner, *Org. Mass Spectrom.*, 11 (1976) 741.
10 L. Crombie and W. M. L. Crombie, *Phytochemistry*, 16 (1977) 1413.
11 M. Nordqvist, J.-E. Lindgren and S. Agurell, *Res. Monogr. Ser. Nat. Inst. Drug Abuse (USA)*, 7 (1976) 64.
12 D. J. Harvey, *Biomed. Mass Spectrom.*, 4 (1977) 88.
13 J.-P. Thenot, E. C. Horning, M. Stafford and M. G. Horning, *Anal. Lett.*, 5 (1972) 217.
14 H. Vorbrüggen, *Angew. Chem.*, 75 (1963) 296.
15 R. N. Smith and C. G. Vaughan, *J. Pharm. Pharmacol.*, 29 (1977) 286.
16 H. Budzikiewicz, R. T. Alpin, A. D. Lightner, C. Djerassi, R. Mehoulam and Y. Gaoni, *Tetrahedron*, 21 (1965) 1881.
17 T. B. Vree and N. M. M. Nibbering, *Tetrahedron*, 29 (1973) 3849.
18 J. K. Terlouw, W. Heerma, P. C. Burgers, G. Dijkstra, A. Boon, H. F. Kramèr and C. A. Salemink, *Tetrahedron*, 30 (1974) 4243.
19 T. B. Vree, *J. Pharm. Sci.*, 66 (1977) 1444.
20 V. Claussen, H.-W. Fehlhaber and F. Korte, *Tetrahedron*, 22 (1966) 3535.
21 L. Hanuš, L. Hruban and Z. Krejči, *Acta Univers. Palack. Olomuc.*, 74 (1975) 167.
22 L. Hanuš and Z. Krejči, *Acta Univers. Palack. Olomuc.*, 74 (1975) 161.
23 M. E. Wall, D. E. Brine and M. Perez-Reyes, in S. Szara and M. Braude (Editors) *Pharmacology of Cannabis*, Raven Press, New York, 1975, p. 1.