tabolism in humans. Acetaminophen appears to be excreted in the urine primarily as acetaminophen sulfate when administered to rats in doses comparable to human doses. In contrast, the glucuronide conjugate constitutes the largest fraction of the dose of acetaminophen excreted in the urine by humans. Therefore, results from studies of the interference by other drugs upon acetaminophen conjugation in the rat cannot be readily extrapolated to similar situations in humans. This possibly explains why Ramachander *et al.* (4) observed an interaction between acetaminophen and salicylate in rats while Levy and Regårdh (7) could not demonstrate this in humans.

(1) A. J. Cummings, M. L. King, and B. K. Martin, *Brit. J. Pharmacol. Chemother.*, **29**, 150(1967).

(2) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60**, 215(1971).

(3) H. Büch, K. Pfleger, and W. Rüdiger, *Z. Klin. Chem. Biochem.*, **5**, 110(1967).

(4) G. Ramachander, F. D. Williams, and J. F. Emele, *J. Pharm. Sci.*, **62**, 1498(1973).

(5) B. H. Thomas and B. B. Coldwell, *J. Pharm. Pharmacol.*, **24**, 243(1972).

(6) T. A. Miettinen and E. Leskinen, in "Metabolic Conjugation and Metabolic Hydrolysis," vol. I, W. H. Fishman, Ed., Academic, New York, N.Y., 1970, pp. 170, 171.

(7) G. Levy and C. Regårdh, *J. Pharm. Sci.*, **60**, 608(1971).

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Decomposition of Cannabidiol in Chloroform Solution

Keyphrases □ Cannabidiol—stability in chloroform and ethanol, GLC, TLC □ Stability—cannabidiol in chloroform and ethanol, GLC, TLC

To the Editor:

Concurrent with our investigation of the stability of the cannabinoids, we observed what appeared to be an unusually large loss of cannabidiol when stored in a chloroform solution. Since chloroform is frequently employed in cannabinoid extraction and analysis (1–10), we felt it imperative to investigate the stability of cannabidiol in this solvent. Previous reports on the stability of cannabinoids in solution were concerned primarily with crude marijuana extracts. Kubena *et al.* (11) reported that cannabidiol was one of the cannabinoids present in a 43-year-old alcoholic solution of marijuana extract, and Turner *et al.* (12) recently indicated that the extractable cannabinoids, including cannabidiol, are stable in chloroform for at least 6 days. However, we wish to report a marked decomposition of cannabidiol in chloroform as compared to its relative stability in ethanol.

Table I—GLC Analysis[a] of Cannabidiol in Ethanol and in Chloroform

Day	Cannabidiol Peak Area[b]		Cannabidiol–Steroid Ratio[c]	
	A(Ethanol)	B(Chloroform)	C(Ethanol)	D(Chloroform)
0	100	100	1.00	1.00
1	105	91	1.02	0.88
2	85	61	0.97	0.68
3	80	54	0.96	0.56
4	85	34	0.89	0.31
5	82	23	0.97	0.19
6	86	14	0.96	0.09
7	87	9	0.97	0.04
8	84	5	0.96	0.01

[a] Average of two or three determinations where integrated peak area differences were not greater than 8%. [b] Percent relative to the peak area on Day 0. [c] Normalized to 1.00 on Day 0; actual values on Day 0 were 1.10 for C and 1.05 for D.

Four 1-ml solutions, each containing 1.0 mg of cannabidiol[1], were prepared: Solution A, in absolute ethanol; Solution B, in spectrograde chloroform[2]; Solution C, in absolute ethanol containing 1.0 mg 4-androstene-3,17-dione[3] (steroid) as an internal standard (7–9); and Solution D, in spectrograde chloroform containing 1.0 mg of steroid as an internal standard. Except during analysis, the solutions were stored at room temperature in the dark in tightly stoppered 1-ml volumetric tubes.

The GLC analysis was performed using a gas chromatograph[4] equipped with a digital integrator[5] and dual 0.6-cm (0.25-in.) by 1.8-m (6-ft) glass columns packed with 3% OV-17 on 100–120-mesh Gas Chrom Q. The experimental conditions were: injection port temperature, 232°; detector temperature, 240°; column temperature, 218°; and nitrogen flow rate, 50 ml/min[6]. Dual flame-ionization detectors were employed, and the attenuation was adjusted so that a 1.0-μl sample of each solution produced a cannabidiol peak of approximately 70% full-scale deflection on Day 0.

The data (Table I) show that there was a significant decrease in the cannabidiol content in chloroform, even in the first 24 hr, while the cannabidiol content in ethanol remained essentially unchanged. The GLC data of Solutions A and B represent absolute values of peak areas and are subject to the usual experimental variations[7]. The consistency of the cannabidiol to steroid ratio in Solution C indicates that the cannabidiol content in ethanol is relatively stable and does not decrease as might be concluded from the data for Solution A alone.

The TLC technique employed was that of Korte *et al.* (10). After impregnation with dimethylformam-

[1] Received from Dr. Monique Braude, Department of Health, Education, and Welfare, U.S. Public Health Service, National Institute of Mental Health, Bethesda, Md.
[2] Fisher Scientific, Fair Lawn, N.J.
[3] E. M. Laboratories Inc., Elmsford, N.Y.
[4] Perkin-Elmer model 990.
[5] Infotronics.
[6] Retention times: cannabidiol, 6.1 min; and steroid, 17.1 min.
[7] Absolute peak area stability could not be maintained because of the necessity of daily removal of the columns from the instrument.

ide–carbon tetrachloride (3:2) and air drying, the plates[8] were spotted with 1.0-μl aliquots of the four solutions and standards of Δ^1- and $\Delta^{1(6)}$-tetrahydrocannabinol[1]. Visualization was accomplished by spraying with 0.1% aqueous di-*o*-anisidine tetrazolium chloride[9]. The TLC data paralleled that of the GLC in the disappearance of cannabidiol from the chloroform solutions. Initially, a single orange component (R_f 0.13) was present in all solutions. For 15 successive days, the solutions of cannabidiol in ethanol (A and C) remained unchanged. However, in the case of the chloroform solutions (B and D), the size and color intensity of the spot decreased in the first 24 hr; by the 8th day, the presence of cannabidiol could not be detected in these solutions. Concomitant with the disappearance of cannabidiol was the appearance of a deep-orange spot at the origin.

On the 9th day, a "micro"[10] Duquenois–Levine test (13) was performed on 5.0-μl aliquots of the four solutions. The ethanol solutions (A and C) showed strongly positive results while the two chloroform solutions (B and D) were negative, indicating the absence of cannabidiol or of Δ^1- or $\Delta^{1(6)}$-tetrahydrocannabinol (14). Formation of cannabinol or of Δ^1- or $\Delta^{1(6)}$-tetrahydrocannabinol in the chloroform solutions was not observed in either the GLC or TLC analyses, as determined from the retention and R_f values of authentic samples[1]. However, a new peak (representing less than 1% of the original cannabidiol content) was present near the Δ^1-tetrahydrocannabinol region in the 8th-day gas chromatograms of the chloroform solutions. This peak may correspond to the major pyrolytic product of cannabidiol reported by Kuppers *et al.* (15). In addition, the chloroform solutions developed a deep-yellow color while the ethanol solutions remained colorless throughout the analysis.

Thus, the decomposition of cannabidiol in chloroform solutions, but not in ethanolic solutions, was demonstrated by GLC, TLC, and colorimetric analysis. Caution should be exercised in the use of chloroform as a solvent for prolonged extraction and storage of cannabidiol. Further study on the stability of cannabidiol in other solvents and the elucidation of the nature of the decomposition products is in progress.

[8] Silica gel, 250 μm, on glass, Brinkmann Inc., Westbury, N.Y.

[9] Fast Blue Salt B, E. M. Laboratories.

[10] One hundred microliters of Duquenois reagent, followed by 5 drops of concentrated hydrochloric acid, is added to the residue from the 5-μl aliquots in a 2-ml glass vial. Within 3 min, a pinkish-violet color is produced. Then 200 μl of chloroform is added with stirring; a violet color in the organic layer indicates a positive test for cannabinoids.

(1) K. D. Parker, J. A. Wright, A. F. Halpern, and C. H. Hine, *Bull. Narcotics*, **20**, 9(1968).

(2) A. E. Robinson, *ibid.*, **23**, 37(1971).

(3) H. M. Stone and H. M. Stevens, *J. Forensic Sci. Soc.*, **9**, 31(1969).

(4) R. A. de Zeeuw and J. Wijsbeek, *Science*, **175**, 778(1972).

(5) P. S. Fetterman, N. J. Doorenbos, E. S. Keith, and M. W. Quimby, *Separatum Experientia*, **27**, 988(1971).

(6) P. S. Fetterman, E. S. Keith, C. W. Waller, O. Guerrero, N. J. Doorenbos, and M. W. Quimby, *J. Pharm. Sci.*, **60**, 1246(1971).

(7) C. E. Turner and K. W. Hadley, *ibid.*, **62**, 251(1973).

(8) *Ibid.*, **62**, 1083(1973).

(9) C. E. Turner, K. W. Hadley, P. S. Fetterman, N. J. Doorenbos, M. W. Quimby, and C. Waller, *J. Pharm. Sci.*, **62**, 1601(1973).

(10) F. Korte, H. Sieper, and S. Tira, *Bull. Narcotics*, **26**, 35(1965).

(11) R. K. Kubena, H. Barry, III, A. B. Segelman, M. Theiner, and N. R. Farnsworth, *J. Pharm. Sci.*, **61**, 144(1972).

(12) C. E. Turner, K. W. Hadley, and K. H. Davis, Jr., *Acta Pharm. Jugoslav.*, **23**, 89(1973).

(13) W. P. Butler, *J. Ass. Offic. Agr. Chem.*, **45**, 597(1962).

(14) C. G. Pitt, R. W. Hendron, and R. S. Hsia, *J. Forensic Sci.*, **17**, 693(1972).

(15) F. J. E. M. Kuppers, R. J. J. C. Lousberg, C. A. L. Bercht, C. A. Salemink, J. K. Terlouw, W. Heerma, and A. Laven, *Tetrahedron*, **29**, 2797(1973).

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