Table I—Peroxide Concentrations in Polyethylene Glycols

Manufacturer	Polyethylene Glycol 300[a]	Polyethylene Glycol 400[a]	Polyethylene Glycol 1500[b]	Polyethylene Glycol 6000 Distearate[b]
I	1.4	3.24	<0.01	1.97
II	4.86	3.95	4.26	N.T.[c]
III	9.3	5.7	N.T.	1.92[d]

[a] Microequivalents of thiosulfate per milliliter of glycol. [b] Microequivalents of thiosulfate per gram of glycol. [c] N.T. = not tested. [d] Additional vendor.

centration of peroxide. The presence of water in the formulation helped prevent any further production of peroxide.

Some of these observations were substantiated by McKenzie (2), who studied peroxide formation and decomposition in triethylene glycol. Azaz *et al.* (3) employed antioxidants to stabilize benzocaine hydrochloride in cetomacrogol solutions containing peroxide impurities.

The reaction of trace metals in the polyethylene glycols with propyl gallate to form colored reactants was prevented by the inclusion of a small quantity of a chelating agent, *e.g.*, 20 ppm of ethylenediaminetetraacetic acid. Since all polyethylene glycols used in these studies contained less than 0.01% ethylene oxide, it is suggested that the reported (1) instability of tripelennamine hydrochloride in polyethylene glycol 300 may have been due not only to the presence of ethylene oxide in the formulation but also the presence of peroxides.

We recommend the use of only the highest quality polyethylene glycols in formulations, a determination of the stability of the active constituent in glycols under elevated temperature conditions (to assess the effect of pretreating the vehicle with antioxidants at elevated temperatures), and, finally, the incorporation of a small percent of water in the formulation.

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Constituents of *Cannabis sativa* L. IX: Stability of Synthetic and Naturally Occurring Cannabinoids in Chloroform

Keyphrases □ *Cannabis sativa* L.—stability of synthetic and naturally occurring cannabinoids in chloroform □ Cannabinoids—stability in chloroform □ Marijuana—stability of synthetic and naturally occurring cannabinoids in chloroform □ Stability—synthetic and naturally occurring cannabinoids in chloroform

To the Editor:

A report from these laboratories demonstrated that chloroform was a more efficient solvent for extracting cannabinoids from *Cannabis sativa* L. than benzene, pentane, hexane, petroleum ether, ethanol, acetone, and ether. Moreover, cannabinoids[1] extracted with chloroform were stable at ambient temperature for 144 hr (1).

Recently, Parker *et al.* (2) reported that synthetic cannabidiol was unstable in spectrograde chloroform over an 8-day period. The authors stated that: "caution should be exercised in the use of chloroform as a solvent for prolonged extraction and storage of cannabidiol." Since members of this research group (3–6) and others (7) have employed chloroform as an extracting solvent and since a working group, sponsored by the United Nations[2], on the chemistry of *Cannabis* and its components recently recommended that the procedure developed in these laboratories be used worldwide, it seemed imperative that additional data be presented in support of chloroform as the solvent of choice for extracting cannabinoids from *Cannabis*.

The basic procedure utilized in these laboratories is as follows. Samples of *Cannabis* are extracted at ambient temperature with nanograde chloroform[3] for 1 hr[4]. After that time, the chloroform is removed *in vacuo* and an ethanolic solution containing a known amount of the internal standard, androst-4-ene-3,17-dione, is added. Therefore, by allowing 15 min for workup, each sample is exposed to chloroform for a maximum of 75 min. Thus, if synthetic and naturally occurring cannabinoids are stable in chloroform for 75 min, chloroform, as previously recommended (1, 7), would be the solvent of choice for extraction of naturally occurring cannabinoids found in crude drug preparations from *C. sativa* L.

We wish to report results of a 3-month stability study using chloroform as the solvent for the following: (*a*) synthetic cannabidiol, (−)-Δ^8- and Δ^9-*trans*-tetrahydrocannabinols, $\Delta^{9,11}$-tetrahydrocannabinol (exocyclic), and cannabinol; (*b*) an extract of female Mexican *Cannabis* grown in Mississippi (coded CMEF-71, ME-A); and (*c*) a synthetic mix-

[1] Combination cannabidiol–cannabichromene, (−)-Δ^9-*trans*-tetrahydrocannabinol, and cannabinol.

[2] United Nations Document MNAR/9/1974.

[3] Mallinckrodt Chemical. One-gallon amber bottles are kept at ambient temperature, opened one at a time as needed, and used with minimum exposure to air, *etc.*

[4] For details, see Refs. 1 and 4–6. Androst-4-ene-3,17-dione was first used as an internal standard by Davis *et al.*, *Lloydia*, **33**, 453(1970).

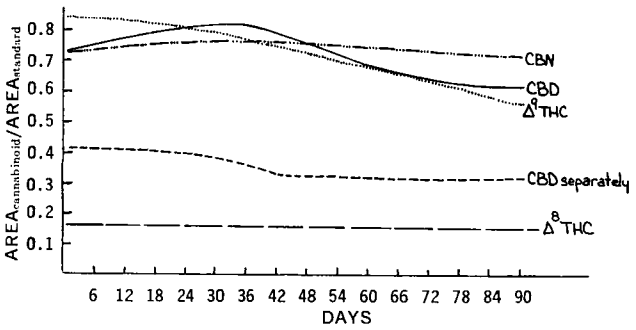

Figure 1—*Graph showing the stability of a mixture of the synthetic cannabinoids [cannabinol, (−)-Δ⁸- and (−)-Δ⁹-trans-tetrahydrocannabinols, and cannabidiol] stored in chloroform over 90 days. Cannabidiol stored separately in chloroform is also presented. The results reported are the area of the cannabinoid divided by the area of the internal standard* versus *the time in days. (THC = tetrahydrocannabinol, CBD = cannabidiol, and CBN = cannabinol.)*

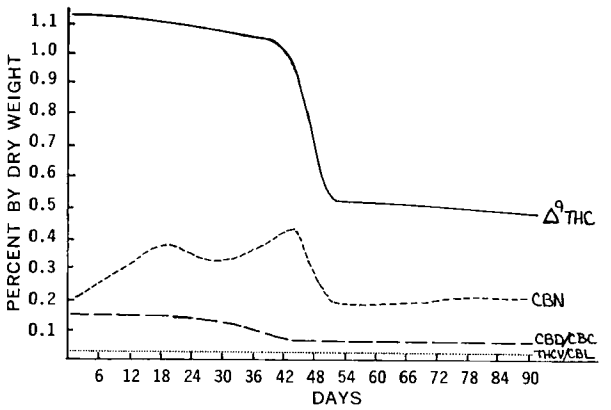

Figure 2—*Graph showing the concentrations of an extract of female Mexican* Cannabis *grown in Mississippi and stored in chloroform for 90 days. The concentrations (percent by dry weight) are plotted against the time in days. [THCV/CBL = a mixture of (−)-Δ⁹-trans-tetrahydrocannabidivarin and cannabicyclol, CBD/CBC = a mixture of cannabidiol and cannabichromene, CBN = cannabinol, and Δ⁹THC = tetrahydrocannabinol.]*

ture of cannabidiol, (−)-Δ⁸- and Δ⁹-*trans*-tetrahydrocannabinols, and cannabinol.

Each chloroform solution was maintained at ambient temperature for 21 days. During this time, each solution was exposed to natural and artificial light. Due to consistent solvent evaporation, each solution was subsequently stored at 6° for the remainder of the experiment. Each sample contained a known amount of the internal standard, and results are reported as the area of the cannabinoid peak divided by the area of the internal standard peak *versus* time in days.

The area under the curve was calculated originally by peak height times width at half-height and was replicated with a GLC–computer system as previously reported (8). GC conditions also have been reported (1, 3–6, 8).

Figure 1 depicts graphically the stability of synthetic cannabidiol as well as a synthetic mixture of cannabidiol, (−)-Δ⁸- and Δ⁹-*trans*-tetrahydrocannabinols, and cannabinol stored in chloroform. Data from these laboratories show that synthetic cannabidiol in chloroform is stable within experimental error for 21 days. The stability diminishes slightly from Day 21 to Day 41 and thereafter remains constant for a 90-day period. With the synthetic mixture, the peak of cannabidiol showed an increase from Day 1 to Day 34, followed by a gradual decline. The slight increase observed for cannabidiol in the synthetic mixture may have been due to an artifact. When synthetic cannabidiol, cannabinol, and (−)-Δ⁸- and Δ⁹-*trans*-tetrahydrocannabinols are mixed and subsequently subjected to GLC analyses, a peak not observed in chromatograms from their respective individual analyses is consistently observed.

The stability of cannabinol and (−)-Δ⁸-*trans*-tetrahydrocannabinol was constant within experimental error in the synthetic mixture over 90 days. However, some diminution in the area under the (−)-Δ⁹-*trans*-tetrahydrocannabinol peak was observed and may have been responsible for the very slight increase in the amount of cannabinol. However, this increase in cannabinol does not account for the total loss of (−)-Δ⁹-*trans*-tetrahydrocannabinol. These data support the Turner *et al.* (6) statement that (−)-Δ⁹-*trans*-tetrahydrocannabinol does not decompose quantitatively to cannabinol.

Figure 2 concerns an extract from female Mexican *C. sativa* grown in Mississippi (coded CMEF-71), which contains a mixture of cannabidiol and cannabichromene, and a mixture of (−)-Δ⁹-*trans*-tetrahydrocannabidivarin and cannabicyclol, as well as cannabinol and (−)-Δ⁹-tetrahydrocannabinol. In these laboratories, (−)-Δ⁸-*trans*-tetrahydrocannabinol was not observed in fresh plant samples when careful analytical parameters were used. (−)-Δ⁹-*trans*-Tetrahydrocannabinol showed a very slight decrease from Day 1 through Day 38 and then underwent a precipitous decrease from Day 44 to Day 50. From Day 50 to Day 90, it once again was relatively stable.

As shown in Fig. 2, cannabinol stability is erratic. There is no lucid interpretation of this behavior. Cannabinol increased from a concentration of 0.2% at Day 1 to a concentration of approximately 0.35% at Day 19. The cannabinoid then began a decline to Day 26, where it again ascended to a high of approximately 0.4% concentration on Day 44. From Day 44 to Day 50, cannabinol decreased once again to approximately 0.2% and then remained fairly constant from that time on.

Turner *et al.* (6) reported a similar decomposition pattern for (−)-Δ⁹-*trans*-tetrahydrocannabinol in dry female Mexican *Cannabis* after storage at 37° for 60 weeks. It was postulated that a free radical moiety might be responsible for the precipitous decomposition.

The combined peak for cannabichromene and cannabidiol was stable from Day 1 through Day 19. Slight decomposition then began until Day 44, after which time it remained stable. This decrease was probably due to the decomposition of cannabichromene. Mass spectral data and data from the 6% OV-1 column have shown that in this particular variant the area under the peak classically labeled cannabidiol is approximately 85% cannabichromene and only 15%

cannabidiol. Cannabichromene is a relatively unstable molecule and decomposes quite readily when exposed to heat, light, and acidic and basic conditions (9). Combined (−)-Δ^9-*trans*-tetrahydrocannabidivarin and cannabicyclol was constant until Day 20. It then declined very little and very slowly. Cannabigerol, although not shown in Fig. 2 due to its small concentration, was very stable.

The data in Fig. 2 cannot be easily explained. Certain phenomena have been reported (9) regarding the ambiguity of cannabichromene decomposing to cannabicyclol. Other than a solvent effect and a "biophysical" parameter not yet defined, this group can offer no explanation of most intrinsic data in Fig. 2.

Individual synthetic cannabinoids and a mixture of synthetic cannabinoids are stable in chloroform and do not decompose in these laboratories, as Parker *et al.* (2) reported for cannabidiol. The instability reported by Parker could be due to impure solvents. We have observed unusual stability problems with certain batches of chloroform. These problems were circumvented by using nanograde and certain brands of spectrograde chloroform[5]. Thus, our data indicate cannabinoids, synthetic or naturally occurring, to be stable in chloroform within experimental error for much longer time periods than are required in a routine analysis. We do, however, agree with Parker *et al.* that cannabinoids should not be stored in chloroform for a prolonged period. Therefore, from published data (1) and data contained within, chloroform remains the solvent of choice for extracting cannabinoids from *Cannabis* preparations.

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Synthetic standards were supplied by the National Institute on Drug Abuse.

[x] To whom inquiries should be directed.

[5] Mallinckrodt and Analabs solvents have given consistently good performance in our laboratory.

Disposition of *N,N*-Bis(phenylcarbamoylmethyl)dimethyl Ammonium Chloride in the Rat: An Interesting Example of First-Pass Metabolism

Keyphrases □ *N,N*-Bis(phenylcarbamoylmethyl)dimethyl ammonium chloride—disposition in rat, first-pass metabolism □ Metabolism, first pass—*N,N*-bis(phenylcarbamoylmethyl)dimethyl ammonium chloride in rat □ Lidocaine derivatives—disposition of *N,N*-bis(phenylcarbamoylmethyl)dimethyl ammonium chloride in rat, first-pass metabolism

To the Editor:

It has recently been demonstrated that the disposition of *N,N*-bis(phenylcarbamoylmethyl)dimethyl ammonium chloride (I), a quaternized lidocaine derivative with antiarrhythmic activity, is dependent on the route of administration in the rat (1). The present report is concerned with a quantitative estimate of the degree to which I is subjected to a first-pass effect in the rat and with a quantitative comparison of the predicted *versus* the observed disposition of the drug after intraperitoneal administration.

After an intravenous (tail vein) dose of 2.5 mg of ^{3}H-I to Sprague–Dawley rats, 35% of the administered radioactivity was ultimately excreted in the urine in the form of I and a carboxylic acid metabolite (II). Radiochromatogram scans of urine samples subjected to high voltage electrophoresis, in addition to reverse isotope dilution data, indicated that about 70% of the total radioactivity in the urine could be accounted for by I.

With intravenous administration of an equal dose to rats with ligated bile ducts, 80% of the radioactive dose was found in the urine. Since the observed increase in urinary excretion of tritium in bile duct-ligated rats may be attributed to "spill over" into the urine of drug and/or metabolite normally excreted in bile, it was estimated that in normal rats 45% of the administered radioactivity is excreted in the bile. Reverse isotope dilution of bile samples revealed less than 5% intact drug (I), while radiochromatogram scans of electrophoregrams of bile samples indicated that a single compound (II) accounted for about 80% of the total radioactivity in the bile. The data are reasonably consistent with Scheme I, where F_s indicates the fraction of administered radioactivity reaching the systemic circulation; f_m, f_u, and f_y represent the fractions of intact drug reaching the systemic circulation that are converted to metabolite, excreted as such, and unaccounted for, respectively; and f_b' and f_u' denote the fractions of the total amount of formed metabolite that undergo biliary and urinary excretion, respectively. The parenthetical values represent percent of administered radioactivity.

Upon intraperitoneal injection of a 2.5-mg dose of ^{3}H-I to normal Sprague–Dawley rats, one finds significantly less tritium ultimately excreted in the urine than is observed after tail vein injection (22%