Table II—Intragastric Administration of 1296 mg of Aspirin

Dog	AUC after 4 hr, hr × mg/liter: AUC–Trapezoid Intermittent Sampling Method	AUC–Integrated Concentration Constant Withdrawal Method
1	555	568
2	492	492
3	491	590
4	425	513
5	717	856
6	522	590
Mean ± 1 *SD*	554 ± 100	601 ± 131
	$p < 0.05$	

the constant blood withdrawal method is mainly due to the peak plasma level observed during the initial period, the difference may become negligible when the plasma concentration–time curve does not contain a peak at the initial period. Table II contains the results of a comparison between the *AUC*–trapezoid determined by the intermittent sampling method and the *AUC*–integrated concentration determined by the nonthrombogenic constant blood withdrawal method after intragastric administration of aspirin. As expected, the difference was not as great as the difference observed in the previous experiment, but it was still significant ($p < 0.05$, paired t test). The plasma concentration–time curve of salicylic acid after intragastric administration of aspirin did not contain the sharp early peak seen after intravenous administration. Despite the gradual increase in the plasma salicylic acid level, the *AUC* was not adequately described by the multiple sampling method.

When the shape of the plasma concentration–time curve is of interest, the collection of blood for the constant blood withdrawal method can be interrupted at predetermined intervals. Therefore, a series of integrated concentrations can be obtained by determining the plasma concentration during each constant blood withdrawal interval. The use of an interruption method increases the number of determinations of the plasma drug concentration, thus reducing one advantage of uninterrupted continuous withdrawal. The advantages of this approach were discussed previously (9).

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Constituents of *Cannabis sativa* L. XIII: Stability of Dosage Form Prepared by Impregnating Synthetic (−)-Δ^9-*trans*-Tetrahydrocannabinol on Placebo *Cannabis* Plant Material

GLENDA S. LEWIS and CARLTON E. TURNER ˣ

Received May 23, 1977, from the *Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, MS 38677.* Accepted for publication October 6, 1977.

Abstract □ Synthetic (−)-Δ^9-*trans*-tetrahydrocannabinol impregnated on placebo *Cannabis* decomposed only 6.3% after being stored for 1 year at −18°. Storage at 5° and room temperature under various conditions led to severe decomposition. The amount of cannabinol observed when (−)-Δ^9-*trans*-tetrahydrocannabinol decomposed indicates that cannabinol is not the only decomposition product.

Keyphrases □ *Cannabis sativa* L.—stability of dosage form prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material □ Stability—dosage form prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material □ (−)-Δ^9-Tetrahydrocannabinol—impregnated on placebo *Cannabis* plant material, stability of dosage form □ Dosage forms—prepared by impregnating synthetic (−)-Δ^9-tetrahydrocannabinol on placebo *Cannabis* plant material, stability

The potency of *Cannabis* preparations varies significantly according to cannabinoid ratios (1). Recently, to overcome these variations, researchers have used placebo *Cannabis* plant material impregnated with synthetic (−)-Δ^9-*trans*-tetrahydrocannabinol (I). However, this procedure also has disadvantages: (*a*) several months are required for the simplest preclinical or clinical study, (*b*) storage conditions in laboratories are not standardized, (*c*) several solvent systems may be used in the impregnation procedure, and (*d*) synthetic I and I in natural *Cannabis* preparations decompose at different rates under different storage conditions (2, 3).

In view of the disadvantages and other considerations[1], this paper reports findings on the stability of a dosage form prepared by impregnating synthetic I on placebo *Cannabis* plant material.

[1] The authors are under contract from the National Institute on Drug Abuse to provide various *Cannabis* preparation to researchers.

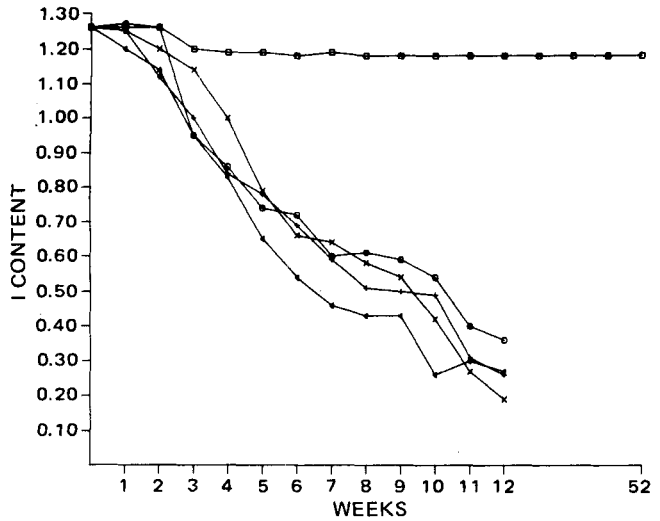

Figure 1—*Decomposition rate of (−)-Δ⁹-trans-tetrahydrocannabinol under different storage conditions. Key: □, freezer at −18°; ○, refrigerator at 5°; ▲, room temperature (25°) under nitrogen and exposed to laboratory light; +, room temperature (25°) uncapped and exposed to air and laboratory light; and ×, room temperature (25°) in the dark and exposed to air.*

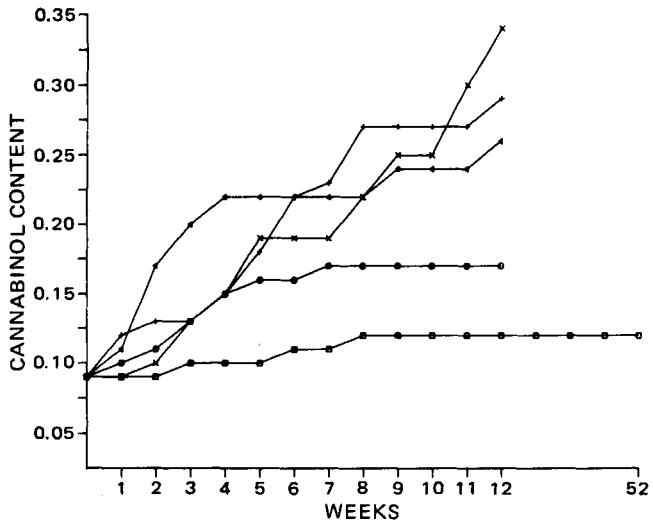

Figure 2—*Cannabinol content resulting from the decomposition of (−)-Δ⁹-trans-tetrahydrocannabinol. Storage conditions were the same as those reported in Fig. 1.*

EXPERIMENTAL

Preparation of Placebo—Manicured[2] *Cannabis sativa* L. (300 g) from an Indian variant[3] grown in Mississippi was extracted with hexane (8 × 1.5 liters) to provide a placebo containing trace amounts of cannabinoids.

Impregnation of Placebo with I—To 300 g of placebo *Cannabis* in an indented 3-liter, round-bottom flask was added 6 g of synthetic I in 500 ml of petroleum ether (bp 60–80°). The flask was attached to a rotary evaporator[4] and rotated vigorously *in vacuo* under mild heat until the solvent was removed. Rotation continued until the placebo material no longer lumped and/or adhered to the walls of the flask. The impregnated material was then analyzed in triplicate and was shown to be coated homogeneously with 1.26% (w/w) of I[5].

The coated material was then bottled in wide-mouth amber glass bottles sealed with plastic caps with a paper lining and stored under the following conditions: (*a*) in the freezer at −18°, (*b*) in the refrigerator at 5°, (*c*) at room temperature (25°) under nitrogen and exposed to laboratory light, (*d*) at room temperature (25°) uncapped and exposed to air and laboratory light, and (*e*) at room temperature (25°) in the dark and exposed to air. Ethanol, ether, methylene chloride, and chloroform were unsatisfactory in impregnating I on placebo material. Analytical data obtained by GLC were so inconsistent that no dosage forms could be prepared.

RESULTS AND DISCUSSION

Turk *et al.* (4) reported that a sample of 100% synthetic I at 0° decomposed to cannabinol (II) in the ratio of 97.1:2.9% over 5 months. Razdan *et al.* (5) reported that synthetic I oxidized to II at the rate of 10%/month at 25°. These data suggest II as the only decomposition product of synthetic I, but Turner *et al.* (2) reported that the amount of II plus I in stored plant material does not account for the original amount of I present. This report suggested the possibility of unidentified cannabinoids being intermediates in the decomposition of I to II and/or that naturally occurring I, when stored in plant material, may decompose by a different pathway. Thus, it seemed imperative to observe the ratios of I, II, and other cannabinoids in this study.

The placebo *Cannabis* prepared contained trace amounts of cannabidiol (III) and cannabichromene (IV), 0.02% I, and 0.02% II. After impregnating the placebo material with synthetic I, the cannabinoid content was: I, 1.26%; II, 0.09%; and III plus IV, trace (<0.01%) amounts.

Samples from each storage condition were anlayzed every week for 12 weeks; after that time, the lowest value for I observed (Fig. 1) was 0.19% in samples stored in the dark at room temperature. Uncapped samples at room temperature exposed to air and light and samples stored at room temperature under nitrogen and exposed to laboratory light contained 0.26 and 0.27%, respectively. The samples stored in the refrigerator contained 0.36% of I. Thus, the decomposition rate for these storage conditions varied from 85 to 71%. No available data explain why samples stored at room temperature in the dark and exposed to air decompose at a faster rate than samples stored under nitrogen and exposed to light at the same temperature. This unexpected observation occurred in a replication.

Samples stored in the freezer at −18° decomposed 6.3% at the end of 12 weeks. Due to the excessive decomposition of all other samples, only the samples at −18° were observed further. These samples proved stable with no additional decomposition up to 52 weeks. In natural *Cannabis* plant material, I stored at −18° decomposed at a rate of 3.83%/year (2).

The data indicate that placebo material, impregnated as described and stored at −18°, is stable for at least 1 year and can be used in experimental designs. Impregnated *Cannabis* plant material not stored under freezer conditions should not be used, and any data published on *Cannabis* impregnated with I where storage under freezer conditions was not specified are suspect at best. Moreover, temperature seems to be the most critical factor in initiating the decomposition of I.

Data on the ratios of I and II are presented in Figs. 1 and 2, respectively. When observing these ratios in samples stored at −18°, it is impossible to state that II is not the only decomposition product of I. However, in all other samples, I decomposed drastically with no corresponding increase in II. For example, in samples stored under nitrogen and light at room temperature, I decreased from 1.26 to 0.27% and II increased from 0.11 to 0.26%. This result strongly indicates that additional products are formed in the decomposition mechanism of I to II and does not support previous reports (4, 5). This point was supported recently when two new cannabinoids were isolated and characterized from an extract of *Cannabis:* (+)-cannabitriol and (−)-10-ethoxy-9-dihydroxy-$\Delta^{6a(10a)}$-tetrahydrocannabinol (6). Neither cannabinoid was detectable by routine GLC; however, by using the silylation method described by Turner *et al.* (7), both compounds were detectable by GLC.

Furthermore, new unidentified cannabinoids of the cannabitriol type are currently being investigated as possible products formed when synthetic or naturally stored I decomposes. Since confusion exists as to the mechanistic pathway from I to II and since most active metabolites of I are hydroxylated, the cannabitriol-type molecules may be present in natural *Cannabis* and placebo *Cannabis* impregnated with I and may be responsible for much of the unexplained pharmacological data on *Cannabis* products (8).

[2] Manicured material is devoid of seeds and large stems; manicuring is accomplished by passing the material through a 10-mesh sieve.

[3] Seed Code IN-B, original seed obtained from Dr. C. K. Atal, Regional Research Laboratory, Jammutawi, India.

[4] Buchi/Brinkmann Rotavapor-R.

[5] Analyses were performed as reported by C. E. Turner, K. W. Hadley, J. H. Holley, S. Billets, and M. L. Mole, Jr., *J. Pharm. Sci.*, **64**, 810 (1975).

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Metabolism and Excretion of Normorphine in Dogs

S. Y. YEH ˣ, R. L. McQUINN *, H. A. KREBS, and C. W. GORODETZKY

Received August 19, 1977, from the *National Institute on Drug Abuse, Division of Research, Addiction Research Center, Lexington, KY 40511.* Accepted for publication October 3, 1977. *Present address: College of Pharmacy, University of Kentucky, Lexington, KY 40506.

Abstract □ Normorphine metabolism was studied in dogs given 20 mg of normorphine hydrochloride/kg sc. Free and conjugated normorphine excreted in the urine over 144 hr represented 32 and 32%, respectively, of the administered dose. Eighty percent of the urinary excretion of the drug occurred within 9 hr. One percent of the administered dose was excreted as free normorphine in the feces. The urine was chromatographed on a column. Evaporation of the washing and methanolic effluent yielded a residue, which was purified by crystallization from aqueous methanol. Results of UV and IR studies, elemental analysis, and determination of normorphine and glucuronic acid content established the identity of this metabolite as normorphine 3-glucuronide. Dihydronormorphine and dehydronormorphine were detected with GLC–mass spectrometry as minor metabolites.

Keyphrases □ Normorphine—metabolism and excretion in dogs □ Metabolism—normorphine in dogs □ Excretion—normorphine in dogs □ Narcotic analgesics—normorphine, metabolism and excretion in dogs

A normorphine conjugate was observed in the urine of humans and laboratory animals after administration of normorphine, morphine, and codeine (1–6), but its nature has not been characterized. The excretion of normorphine in the dog, the isolation and characterization of the normorphine conjugate as normorphine 3-glucuronide, and the detection of dihydronormorphine and dehydronormorphine as minor metabolites are discussed in this report.

EXPERIMENTAL

Materials and Methods—Normorphine hydrochloride[1], 20 mg/kg sc, was injected into two female dogs deprived of food for 24 hr and hydrated by intubation with about 500 ml of water. The dogs were housed in individual metabolic cages, and urine and feces were collected for 144 hr.

Normorphine was determined by GLC in the urine and feces, with and without hydrolysis, as described (4). Specimens were hydrolyzed with acid or β-glucuronidase[2] (4). Free and total normorphine were estimated from unhydrolyzed and hydrolyzed specimens, respectively. Conjugated normorphine was calculated as the difference between the total and the free drug.

GLC–Mass Spectrometry—Chemical-ionization mass spectral data were obtained on a gas chromatograph–mass spectrometer[3] as described (7). The mass fragment pattern of obvious GLC peaks was analyzed. In addition, the whole chromatogram was scanned for possible normorphine metabolite ions such as morphine, norcodeine, *N*-hydroxynormorphine, dihydronormorphine, and monohydroxynormorphine.

Isolation of Normorphine Metabolites—An aliquot of the first 24-hr urine was centrifuged and passed through a resin[4] column (2.1 × 40 cm). The column was washed with 150 ml of distilled water and eluted with 300 ml of 2.5% NH_4OH in methanol. The water washing and the methanol eluate were separately evaporated to syrupy residues under reduced pressure in a water bath at 50°. The residues were suspended in 20 ml of 2% NH_4OH and extracted three times with 1,2-dichloroethane containing 30% 2-propanol. The extracts were concentrated for identification of free normorphine metabolites. The aqueous phase was used to isolate conjugated normorphine as described below.

An aliquot of the free base fraction along with authentic normorphine was chromatographed on instant TLC sheets impregnated with silica gel[5], with ethyl acetate–methanol–ammonium hydroxide (17:2:1) as the developing solvent. After a portion of the chromatogram was sprayed with iodoplatinate, three spots appeared with R_f values of 0.6 (pinkish), 0.73, which corresponds to authentic normorphine (bluish purple), and 0.95 (bluish purple). The chromatogram was sectioned horizontally according to the zones 0–75 (R_f 0.3), 76–110 (R_f 0.6), 111–140 (R_f 0.73), and 141–155 (R_f 0.95) mm and eluted with methanol. The eluates were concentrated and analyzed by GLC.

The aqueous phase was evaporated to a syrupy residue. The residue was triturated with methanol to yield methanol-soluble and methanol-insoluble fractions. The methanol-insoluble fraction was recrystallized from aqueous methanol and dried under vacuum, and it yielded 50 mg of snow-white crystalline material. Approximately 60% of the conjugate was isolated from the water washing fractions.

The methanol-soluble fraction was chromatographed on instant TLC sheets impregnated with silica gel[5], with 1-butanol–acetic acid–water (35:3:10) (Solvent A) as the developing solvent. A section of the chromatogram was sprayed with iodoplatinate solution and showed four spots at R_f 0.0, 0.33, 0.6, and 0.9. The chromatogram was sectioned horizontally according to zones 0–50 (R_f 0.33), 51–120 (R_f 0.6), and 121–150 (R_f 0.9) mm and eluted with water. The eluate was concentrated, hydrolyzed with

[1] Merck Sharp & Dohme, Philadelphia, Pa.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Finnigan model 3300.
[4] Amberlite XAD-2 resin, Rohm & Haas Co., Philadelphia, Pa.
[5] Gelman Instrument Co., Ann Arbor, Mich.