REFERENCES

(1) R. I. Poust and V. F. Smolen, *J. Pharm. Sci.*, **59**, 1461 (1970).

(2) V. F. Smolen and R. I. Poust, *ibid.*, **60**, 1745(1971).

(3) V. F. Smolen and F. P. Siegel, *ibid.*, **57**, 378(1968).

(4) V. F. Smolen and L. D. Grimwood, *J. Colloid Interface Sci.*, **36**, 308(1971).

(5) V. F. Smolen, D. E. Snyder, and R. J. Erb, *J. Pharm. Sci.*, **59**, 1093(1970).

(6) V. F. Smolen, *Amer. J. Pharm. Educ.*, **33**, 381(1969).

(7) V. F. Smolen and E. J. Williams, *J. Pharm. Sci.*, **61**, 921 (1972).

(8) D. S. Riggs, "The Mathematical Approach to Physiological Problems," Williams & Wilkins, Baltimore, Md., 1963, pp. 120–168.

(9) V. F. Smolen, *J. Pharm. Sci.*, **60**, 354(1971).

(10) R. D. Schoenwald, Ph.D. thesis, Purdue University, Lafayette, Ind., 1971.

(11) E. Ackerman and J. B. Hazelrig, U. S. Atomic Energy Commission Symposium No. 3, June 1964.

(12) A. R. Cade, *Soap Sanit. Chem.*, **26**, 35(1950).

(13) A. R. Cade, *J. Soc. Cosmet. Chem.*, **2**, 281(1951).

(14) P. B. Price, *Ann. Surg.*, **134**, 476(1951).

(15) G. N. Ling and M. H. Krumash, *J. Gen. Physiol.*, **50**, 677(1967).

(16) M. M. Breuer, *J. Phys. Chem.*, **68**, 2067(1964).

(17) *Ibid.*, **68**, 2074(1964).

(18) *Ibid.*, **68**, 2081(1964).

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Oral and Parenteral Formulations of Marijuana Constituents

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Abstract □ The lack of detailed information on the manipulation and preparation of cannabinoid formulations prompted an investigation of useful vehicles for administration of tetrahydrocannabinols and crude marijuana extracts. It was found that pure Δ^9- and Δ^8-tetrahydrocannabinols could be quantitatively handled by chipping samples at 4°, transferring them to cold receptacles for weighing, and, after liquefying the cannabinoid at 50°, adding a warmed vehicle for further transfers and final dilution. Tetrahydrocannabinol samples larger than 10 g. were liquefied at 55° and poured directly into a tared receptacle. Crude marijuana extract samples were smeared on tared receptacles and diluted and transferred as above. Stock solutions of cannabinoid in sesame oil were stable for months and could be used directly for oral administration or for formulating injectables. Suitable emulsions for parenteral use consisted of sesame oil (10–15%) plus polysorbate 80 (0.4–1%) in saline containing up to 4% tetrahydrocannabinol or sesame oil (5–10%) plus polyvinylpyrrolidone (4–5%) containing approximately 1% cannabinoid. Such an approach incorporated an innocuous vehicle, did not require the presence or removal of an organic solvent, provided wide latitude for needed concentrations of cannabinoid, and was timesaving. The ratio of emulsifier and cannabinoid was critical for stable emulsions.

Keyphrases □ Marijuana constituents—oral and parenteral formulations, stability in various solvents, biological evaluation of formulations, vehicle toxicity □ Toxicity, vehicle—stability of marijuana constituents in various solvents, biological evaluation of formulations for long-term oral and parenteral administration to laboratory animals □ Formulations—effect of solvents on marijuana constituent stability, biological evaluation, vehicle toxicity □ Tetrahydrocannabinols—stability in various solvents, biological evaluation of formulations for long-term oral and parenteral administration, vehicle toxicity

A full understanding of the biological properties of marijuana constituents has been hampered by the lack of a desirable vehicle for the preparation of injectables. The diversity of formulations and routes of administration of the active ingredients of marijuana has somewhat complicated the comparison of pharmacological, toxicological, and behavioral data from different laboratories. Those studies involving a single administration have justifiably not been too concerned with the influence of the vehicle. However, chronic investigations at relatively high doses (>50 mg./kg.) of cannabinoids cannot overlook the effect of the diluent.

The tetrahydrocannabinols have been identified as the major biologically active components of marijuana (1, 2). Whereas crude marijuana extracts are of a tar consistency, the tetrahydrocannabinols are highly viscous oils, virtually of a glue nature at room temperature. Despite reports of extensive analytical data to establish the purity of the sample, little detail has been given as to how to transfer and manipulate the compounds to obtain accurate concentrations.

Relatively high concentrations of tetrahydrocannabinol or crude marijuana extract for intragastric use can be achieved in natural vegetable oils (3). On the

Table I—Solubility of Cannabinoids in Various Solvents[a]

Solvent	—Δ⁹-Tetrahydrocannabinol— g./ml.	Appearance	—Δ⁸-Tetrahydrocannabinol— g./ml.	Appearance	—Crude Marijuana Extract— g./ml.	Appearance
Absolute ethanol	1.00	Solution	1.00	Solution	1.00	Solution
Acetone	1.00	Solution	1.00	Solution		
Benzyl alcohol	0.90	Solution	0.91	Solution		
Dimethyl sulfoxide	0.54[b]	Solution	0.62[b]	Solution		
Sesame oil	0.30[c]	Solution	0.30[c]	Solution	0.40[c]	Solution
Propylene glycol	0.58[c]	Solution	0.89[c]	Viscous		
Glycerol	0.39	Viscous	0.38	Viscous		
Polysorbate 80[d]	0.28	Paste	0.22	Paste	0.45	Paste

[a] The limit of solubility was taken as 1 g./ml. of tetrahydrocannabinol in ethanol; see explanation in text. [b] Not continued until maximum solubility. [c] Higher concentrations were too viscous for practical use. [d] Polysorbate 80 is polyoxyethylene monooleate (Tween 80).

other hand, aqueous systems necessary for parenteral administration must incorporate some type of emulsifier. The limitations of such parenteral formulations reside in three considerations: (*a*) the low concentration of cannabinoid achieved dictates the volume of formulation that must be given, (*b*) the concentration of emulsifier and number of treatments introduce the hazard of vehicle toxicity, and (*c*) the lability of the formulation may require frequent preparation of the injectable. One must also keep in mind that formulations developed for animal trials may not be acceptable for human investigations.

The present paper provides sufficient details as to the preparation of dosage forms of tetrahydrocannabinol and crude marijuana extract, outlines the limitations of various injectables, and reviews formulations used in marijuana studies.

EXPERIMENTAL

Cannabinoids—Crude marijuana extract, synthetic (−)-*trans*-Δ⁹-tetrahydrocannabinol, and synthetic (−)-*trans*-Δ⁸-tetrahydrocannabinol were used[1]. Cannabinoid shipments were in approximately 50-g. quantities per bottle, and the crude extract came in 500-g. quantities per bottle. The provided analytical data on all Δ⁹-tetrahydrocannabinol lot numbers showed a purity between 95 and 97% by GC and an optical rotation range of from −163 to −175°. Corresponding data for Δ⁸-tetrahydrocannabinol were 98–99% purity by GC and optical rotation values from −254 to −268°. The crude marijuana extract assayed as 25% Δ⁹-tetrahydrocannabinol, 2–3% cannabidiol, and 2–3% cannabinol.

Cannabinoid Transfer Procedure—Samples of tetrahydrocannabinols between 0.1 and 10 g. were chipped free at 4° with a clean, cold, ice pick and transferred to cold, tared, glass receptacles. An 18 × 60-mm. conical tube was used for samples under 1 g., a 10-ml. conical flask was used for samples between 1 and 3 g., and a 25-ml conical flask was used for samples between 3 and 10 g. Weighings were rapidly performed on an analytical balance at room temperature. The cannabinoid in its receptacle was next placed in a water bath at 45° under a stream of nitrogen until liquefaction occurred (5–10 min.). Ten 50-g. samples of tetrahydrocannabinol were directly liquified in their shipping container by heating the inverted container with an IR lamp to 55° under a canopy of nitrogen whenever possible. The compound was collected in a tared beaker placed in a water bath at 50°. The crude marijuana extract was also directly warmed in its shipping container to 45° in a water bath, and 50–500 g. of liquified material was transferred by pouring into a tared beaker. Samples less than 50 g. were transferred by spatula.

Cannabinoid Solubility Studies—In the solubility trials, 0.1-ml. aliquots of analytical reagent grade ethyl alcohol, benzyl alcohol, acetone, or dimethyl sulfoxide were added stepwise to 1 g. of cannabinoid until maximum solubility was achieved at room temperature. Similar solubility studies were conducted with 0.1-ml. aliquots of sesame oil USP, propylene glycol, and glycerol in conjunction with 1 g. of cannabinoid.

Cannabinoid Solution Preparation—True solutions of cannabinoid were prepared by dissolving 1 g. of liquified tetrahydrocannabinol in 1 ml. of ethanol or acetone. Stock solutions in sesame oil were prepared by dissolving 10 g. of liquified cannabinoid in 25 ml. of warmed sesame oil. For all true solutions, formulations were transferred by pipet or poured into graduate cylinders to accomplish quantitative dilution at room temperature. In this manner, 1–40% solutions of cannabinoids in ethanol and sesame oil were prepared and sealed in vials flushed with nitrogen.

Cannabinoid Emulsion Preparation—One milliliter of 100–400 mg./ml. of tetrahydrocannabinol in sesame oil was introduced into a 50-ml. beaker or conical flask followed by 0.01–0.1 ml. of polysorbate 80[2] and isotonic saline to a final volume of 10 ml. Emulsification was performed by sonication with a sonifier at intensity position 5 at 7.5 amp. for 30 sec.[3]. On occasion, the polysorbate 80 was replaced by 5% polyvinylpyrrolidone in isotonic saline or 1% poloxalene[4]. Other emulsions were formed by addition of 0.1–0.3 ml. of warmed polysorbate 80 to 100–200 mg. of liquified cannabinoid, mixing with a microspatula and mechanical Vortex, and followed by trituration with 1–15 ml. of isotonic saline. Attempted emulsion formation with cannabinoid solutions in organic solvents was performed by trituration of 0.1–0.3 ml. of approximately 50% cannabinoid solutions with approximately 0.9 ml. of steroid-suspending vehicle, 10% dextran[5], 10% mannitol, or 0.9% saline.

Stability Studies—Optical rotation of tetrahydrocannabinols was determined at a concentration of 20 mg./ml. in chloroform with a polarimeter[6]. Suitable aliquots of ethanol solutions were diluted with chloroform to attain the necessary analytical concentration. In the instance of sesame oil formulations, 10–30 ml. was extracted with an equal volume of hexane–4% sodium hydroxide in 50% ethanol. Aliquots of the hexane layer were concentrated and applied to thin-layer silica gel flexible sheets impregnated with dimethylformamide and developed with hexane–benzene–methanol (30:30:4) in a sandwich chamber for 45 min.[7]. The cannabinoids were visualized with Turnbull's blue reagent.

Biological Studies—Potential cannabinoid vehicles were tested in animal trials for clinical toxicity signs. Single treatments of ethanol were given at doses between 0.005 and 0.05 ml./kg. p.o. or i.v. to 6–10 rats, at 2 ml./kg. p.o. to three rhesus monkeys, at 0.45 ml./kg. i.v. to five rhesus monkeys, at 0.75 ml./kg. p.o. to two beagle dogs, and at 0.2 ml./kg. i.v. to three beagle dogs. Chronic treatment with 100% sesame oil was performed at 0.05 ml./kg. p.o. for 119 days in 120 rats, at 1 ml./kg. s.c. for 28 days in three New Zealand rabbits, and at 3 ml./kg. p.o. in eight rhesus monkeys. Sesame oil (10%)–polysorbate 80 (0.4%) dispersed in isotonic saline was administered at 0.3 ml./kg. i.p. for 5 days to three rats, at 0.1 ml./kg. i.v. for 5 days to three rats, and at 6 ml./kg. i.v. for 28 days to two rhesus monkeys. Sesame oil (10%) in 4.5% polyvinylpyrrolidone was tested at 6 ml./kg. i.v. for 28 days in two rhesus

[1] Supplied under contract with the National Institute of Mental Health.

[2] Tween 80.

[3] Branson, model LS75, Heat Systems-Ultrasonics, Inc., Plainview, N. Y.

[4] Pluronic F68, BASF Wyandotte Corp., Wyandotte, Mich.

[5] The dextran was obtained from Bios Laboratories, Inc., New York, N. Y.; particle size not given.

[6] Bendix Corp., Cincinnati, Ohio.

[7] M. Hagopian, Mason Research Institute, Worcester, Mass., unpublished data.

Table II—Characteristics of Aqueous Systems for Dispersing Cannabinoids[a]

Cannabinoid		Solvent		Diluent			Final Concentration of Ingredient (v/v)		
Name	mg.	Name	ml.	Name	ml.	Appearance of Preparation	THC, mg./ml.	Solvent, %	Diluent, %
Δ^8-THC[b]	94	Ethanol	0.2	SSV[b]	0.8	Flocculent	9.4	20	80
Δ^9-THC	105	Ethanol	0.2	SSV	0.8	Flocculent	10.5	20	80
Δ^8-THC	107	DMSO[b]	0.2	SSV	0.8	Flocculent	10.7	20	80
Δ^8-THC	48	Benzyl alcohol	0.1	Dextran, 10%	0.9	Unstable emulsion[c]	4.8	10	90
Δ^8-THC	309	Benzyl alcohol	0.3	Mannitol, 10%	14.7	Two-phase	—	2	98
Δ^9-THC	44	Ethanol	0.1	Dextran, 10%	0.9	Unstable emulsion[c]	4.4	10	90
Δ^8-THC	301	Propylene glycol	0.1	Saline, 0.9%	0.9	Two-phase	—	10	90
Δ^9-THC	104	Propylene glycol	0.1	Saline, 0.9%	0.9	Two-phase	—	10	90
Δ^8-THC	265	Glycerol	0.5	Saline, 0.9%	0.9	Two-phase	—	36	64
Δ^9-THC	292	Glycerol	0.5	Saline, 0.9%	0.9	Two-phase	—	36	64
Δ^9-THC	250	Sesame oil	1.0	PVP[b], 10%	9.0	Stable emulsion[d]	25	10	90
Δ^9-THC	250	Sesame oil	1.0	PVP[b], 5%	9.0	Stable emulsion[d]	25	10	90
Δ^9-THC	250	Sesame oil	1.0	Saline, 0.9%	9.0	Unstable emulsion[e]	25	10	86
	or	+						+	
	150	Polysorbate 80	0.04			Stable emulsion[e]	15	0.4	
Δ^8-THC	90	Polysorbate 80	0.1	Saline, 0.9%	0.9	Stable emulsion	9	10	90
Δ^9-THC	57	Polysorbate 80	0.1	Saline, 0.9%	0.9	Stable emulsion	5.7	10	90
Δ^9-THC	580	Polysorbate 80	0.6	Saline, 0.9%	4.7	Unstable emulsion[f]	100	10	90
Δ^9-THC	3539	Polysorbate 80	1.0	Saline, 0.9%	15.7	Unstable emulsion[f]	180	5.1	95
Δ^9-THC	45	Polysorbate 80	0.5	Saline, 0.9%	10.2	Unstable emulsion[f]	45	4.9	95
Δ^9-THC	162	Polysorbate 80	0.3	Saline, 0.9%	15.8	Stable emulsion	10	1.8	98
Δ^8-THC	170	Polysorbate 80	0.3	Saline, 0.9%	13.8	Stable emulsion	12	2.1	98

[a] The cannabinoid was dissolved in the solvent at 25–40°, and the diluent was added dropwise with mixing; volume of cannabinoid mass above 100 mg. taken into account. [b] THC = tetrahydrocannabinol. SSV = steroid-suspending vehicle (see footnote to Table 1). DMSO = dimethyl sulfoxide. PVP = polyvinylpyrrolidone. [c] Levigation restores emulsion. [d] 5–10% oil separation after several hours, but sonication restores emulsion. [e] Addition of polysorbate 80 and saline to 1 ml. of cannabinoid in sesame oil followed by sonication. [f] Emulsion with less than 10% polysorbate 80 separated within minutes unless cannabinoid concentration was less than 15 mg./ml.

monkeys. Saline solutions of 2.5–10% polysorbate 80 were administered at doses between 0.03 ml./kg. i.v. and 0.3 ml./kg. p.o. to 5–10 rats; at 2–3.6 ml./kg. p.o. and i.v., respectively, to two rhesus monkeys, and at 1 ml./kg. i.v. to two beagle dogs. A 5.5% sodium glycocholate solution was also tested at 2 ml./kg. i.v. for 9 days in two rhesus monkeys. Hematological and clinical chemical parameters were determined by standard procedures and will be the subject of a separate report.

RESULTS

As the tetrahydrocannabinols thawed, they became tacky and glued to glass, metal, or paper surfaces. This difficulty could be circumvented by performing transfers of samples less than 10 g. at 4° and larger samples after liquefaction. Concentrated solutions could be quantitatively transferred if the outside walls of receptacles were also warmed.

Solubility of Cannabinoids in Solvents Yielding Solutions—Because of fragmentary data on the solubility of cannabinoids, some studies on the solubility of Δ^9- and Δ^8-tetrahydrocannabinols and crude marijuana extract were carried out. Tetrahydrocannabinols behave primarily as liquids and are freely miscible with many organic solvents. In such cases the limit of solubility may be taken at that point where the solvent is still in excess. Since the volume contribution by the cannabinoid mass was approximately 80% in the presence of an organic solvent like ethanol, the solubility limit

Table III—Chemical Stability of Cannabinoid Formulations

Cannabinoid	Diluent	Tehahydrocannabinol, mg./ml.	Temperature	Elapsed Time, Days	Optical Rotation in Chloroform	Estimated Percent Purity by TLC[a]
Δ^9-Tetrahydrocannabinol	None	1000	5°	0	−163	—
	None	1000	55°	0.5 hr.	−160	—
	Ethanol	500	5°	0	−164	90
	Ethanol	500	5°	40	−152	80
	Ethanol	500	22°	40	−126	—
	Ethanol	500	5°	90	—	70
	Chloroform	20	22°	21	−163	—
	Chloroform	20	55°	0.5 hr.	−161	—
	Sesame oil	100	5°	60	—	88
	Sesame oil + polysorbate	10	5°	7	—	90
Δ^8-Tetrahydrocannabinol	None	1000	5°	0	−266	—
	None	1000	55°	0.5 hr.	−263	—
	Ethanol	500	5°	0	−263	98
	Ethanol	500	5°	40	−258	98
	Ethanol	500	22°	40	−256	—
	Chloroform	20	22°	21	−260	—
	Chloroform	20	50°	0.5 hr.	−261	—
	Sesame oil	100	5°	60	—	98
	Sesame oil + polysorbate	10	5°	7	—	99

[a] Silica gel flexible sheets impregnated with dimethylformamide and developed with hexane–benzene–methanol (30:30:4).

Table IV—Vehicle-Induced Clinical Changes Observed in Various Species

Vehicle	Species[a]	Route	Dose, ml./kg. × Days	Number Animals Reacting/ Number Animals Treated	Clinical Signs
Ethanol, absolute	Rat, Fischer	p.o.	0.05 × 1	10/10	None
		i.v.	0.01 × 1	5/9	Slight ataxia for 1–2 min.
	Rat, Wistar-Lewis	p.o.	0.01 × 1	7/7	None
		i.v.	0.005 × 1	3/6	Prostration for 2 hr.; slight ataxia for 2–3 min.
	Monkey, Rhesus	p.o.	2.0 × 1	3/3	Drowsiness for 45 min.
		i.v.	0.45 × 1	5/5	None
	Dog, beagle	p.o.	0.75 × 1	2/2	None
		i.v.	0.2 × 1	1/3	Mild intoxication for 20 min.
Sesame oil USP	Rat, Fischer	p.o.	0.05 × 119	60/120	Mild hematologic changes[b]
	Rabbit, New Zealand	s.c.	1.0 × 28	3/3	None
	Monkey, Rhesus	p.o.	3.0 × 90	3/8	Aspiration pneumonitis
Sesame oil, 10%, + polysorbate 80, 0.4%[d]	Rat, Fischer	i.p.	0.3 × 5	3/3	Moderate weight loss
		i.v.	0.1 × 5	3/3	None
	Monkey, Rhesus	i.v.	6.0 × 28	2/2	None
Polysorbate 80, 2.5%[d]	Rat, Fischer	i.v.	0.03 × 1	5/5	None
	Dog, beagle	i.v.	1.0 × 1	2/2	Ataxia, erythema for 35 min.
Polysorbate 80, 5%[d]	Monkey, Rhesus	p.o.	3.6 × 1	2/2	None
		i.v.	2.0 × 1	1/2	Facial congestion only
Polysorbate 80, 10%[d]	Rat, Wistar-Lewis	p.o.	0.3 × 3	5/10	Dehydration, inactivity, mottled fur, one male dead
		i.v.	0.03 × 5	9/9	None
Sesame oil, 10%, + polyvinylpyrrolidone, 4.5%[d]	Monkey, Rhesus	i.v.	6.0 × 28	2/2	Weight loss
Glycocholate, 5.5%	Monkey, Rhesus	i.v.	2.0 × 9	2/2	Severe edema, hemolysis

[a] Body weights were approximately 100–150 g. for rats, 3.5–4.0 kg. for monkeys, and 10–14 kg. for dogs. [b] Slightly increased coagulation time, serum proteins, red blood cells, hemoglobin, and hematocrit. [c] Mild aspiration pneumonitis after 90 days of treatment. [d] Dispersed in isotonic saline.

of cannabinoid in ethanol may be considered as 1 g./ml. (*e.g.*, the ethanol volume was slightly in excess of that of the cannabinoid). Further additions of cannabinoid to 1 ml. of ethanol yielded true solutions, but now the cannabinoid was the solvent and ethanol the solute. In effect, a solution of 0.95 g./ml. of cannabinoid in ethanol could be produced by dissolving 1 ml. of ethanol in 3.2 ml. (4 g.) of cannabinoid.

The data in Table I are presented for the comparison of cannabinoid solubility in various solvents equated to 1 g./ml. of tetrahydrocannabinol in ethanol. Similar solubilities were obtained for Δ^9- and Δ^8-tetrahydrocannabinols and crude marijuana extract in polar solvents. For practical purposes, 100–400 mg. of cannabinoid can be dissolved in 1 ml. of a suitable organic solvent or natural oil like sesame oil.

Characteristics of Emulsions—To explore possible aqueous systems for cannabinoids, various combinations of solvents, emulsifiers, and diluents were examined. These results (Table II) show that small quantitites of solubilizer or emulsifier provided stable emulsions. Relatively high concentrations of cannabinoid with 10% polysorbate 80 yielded emulsions which were stable for a few minutes; as the concentration of cannabinoid was reduced, the physical stability of the emulsion increased. Reduction of the polysorbate 80 content severely restricted the amount of cannabinoid that could be stably emulsified.

Small quantities of organic solvents failed to provide suitable cannabinoid suspensions (*e.g.*, propylene glycol and glycerol formed a two-phase system upon dilution with saline). The most successful emulsions comprised 1-ml. aliquots of stock solutions of cannabinoid in sesame oil (100–400 mg./ml.) to which 0.01–0.1 ml. of polysorbate 80 and 8–9 ml. of isotonic saline were added and emulsified by sonication. Increased amounts of polysorbate 80 permitted higher concentrations of cannabinoid. Other usable emulsions were obtained with cannabinoid in sesame oil and 5–10% polyvinylpyrrolidone or 1% poloxalene[4]. The sesame oil–polyvinylpyrrolidone emulsions were stable for 3–6 hr. when a slight (5–10%) oil slick formed. This oil slick could be readily reemulsified by sonication, and this procedure was successfully applied for several days without deterioration of the emulsion. Concentrations of polyvinylpyrrolidone above 30% when mixed with cannabinoid in sesame oil yielded two-phase systems, and polyvinylpyrrolidone concentrations below 5% formed unstable emulsions. In the instance of poloxalene, concentrations above 2% resulted in unstable emulsions.

Stability of Cannabinoid Formulations—Because of the time required to manipulate the cannabinoid (*e.g.*, chipping, weighing, liquefaction, admixture, and emulsification) and the desire to use stock solutions for long periods of time, some exploration of chemical stability was performed. These data, presented in Table III, reveal that the optical rotations of the tetrahydrocannabinols were not affected by elevated temperatures for short periods of time. In ethanolic solutions at concentrations from 500 to 1000 mg./ml., the optical density of Δ^9-tetrahydrocannabinol decreased about 8% after 40 days at 4°. The decrement was more noticeable over this interval of time at room temperature. Under similar conditions, the

Table V—*In Vivo* Hemolytic Activity of Marijuana Vehicles in Monkeys Treated Intravenously

Number of Monkey and Sex	Vehicle[a]	Dose, ml./kg. × Days	Day in Study	Hemoglobin, g./100 ml.	Serum K^+, meq./l.
1 M + 1 F	Glycocholate	4 × 7	−5	11.6	3.9
			+8	5.5	5.8
1 M + 1 F	Glycocholate	2 × 9	−5	12.9	3.8
			+10	9.2	4.3
1 M + 1 F	Sesame oil–polysorbate 80	6 × 28	−5	12.6	4.5
			+14	11.0	4.5
			+28	11.3	4.4
1 M + 1 F	Sesame oil–polysorbate 80	4 × 28	−5	12.8	4.1
			+14	12.2	4.2
			+28	12.5	4.3
1 M + 1 F	Sesame oil–polysorbate 80	2 × 28	−5	12.5	4.2
			+14	11.2	4.0
			+28	11.7	4.3
1 M + 1 F	Sesame oil–polyvinylpyrrolidone	6 × 28	−2	12.0	4.0
			+11	12.0	4.6
			+25	11.9	4.3
1 M + 1 F	Sesame oil–polyvinylpyrrolidone	4 × 28	−2	12.5	3.9
			+11	9.8	4.6
			+25	12.1	4.3
1 M + 1 F	Sesame oil–polyvinylpyrrolidone	2 × 28	−2	12.2	4.1
			+11	12.4	4.7
			+25	12.7	4.3
1 M + 1 F	Saline	6 × 28	−5	13.0	3.8
			+14	11.5	4.1
			+28	12.6	4.6

[a] Concentrations of vehicles were: glycocholate, 5.5%; sesame oil, 10%; polysorbate 80, 0.4%; polyvinylpyrrolidone, 4.5%; and saline, 0.9%.

optical rotation of Δ^8-tetrahydrocannabinol was virtually unaltered. Dilute solutions of cannabinoid in chloroform were stable for weeks at room temperature or for short periods of heating, as determined by optical rotation. Stock solutions of cannabinoid at 100 mg./ml. in sesame oil and at 10 mg./ml. in 0.4% polysorbate 80-saline were stable for days, as shown by optical rotation and TLC analyses. Interestingly, the black surface material from Δ^9-tetrahydrocannabinol samples gave an optical rotation of −140°. Since optical rotation values diminished instead of increased for Δ^9-tetrahydrocannabinol preparations, little or no conversion of Δ^9-tetrahydrocannabinol to Δ^8-tetrahydrocannabinol took place.

Biological Evaluation of Formulations—*Biological Data on the Oral Route*—Administration of various vehicles used in cannabinoid formulations to several species of laboratory animals provided a basis for evaluating the usefulness of these vehicles (Table IV). Absolute ethanol administered orally produced no clinical changes at the volumes used in rats, monkeys, and dogs. The hazard of synergism or potentiation of cannabinoid effects compromised extensive use of ethanol formulations by any route of administration. Sesame oil administered orally to rats and monkeys for extended periods produced no behavioral changes. However, moderate aspiration pneumonitis due to the gavage technique was observed in most monkeys after 90 days of treatment, while rats exhibited mild increased coagulation times, total serum proteins, circulating red cells, hemoglobin levels, and hematocrit values that were apparently not related to dehydration.

Biological Data on Parenteral Routes—The administration of ethanol intravenously to rats, monkeys, and dogs evoked considerable depression. A vehicle composed of sesame oil (10%) and polysorbate 80 (0.4%) in normal saline administered intraperitoneally and intravenously to rats and intravenously to monkeys produced only moderate weight loss in rats treated intraperitoneally and no observable effects in monkeys. Varying percentages of only polysorbate 80 in normal saline were well tolerated by rats treated intravenously, but dogs and monkeys treated intravenously exhibited erythema and facial congestion. The potential synergism of cannabinoid cardiovascular effects by polysorbate 80 above 2.5% compromises its use in protracted studies. Sesame oil–polyvinylpyrrolidone emulsions induced weight losses in monkeys at 2–6 ml./kg. i.v. A transient, mild hyperkalemia was seen after 11 days of treatment. Sodium glycocholate at 6 ml./kg. i.v. was terminal after a few days in monkeys as a result of hemoglobinuric nephrosis, and 4 ml./kg. i.v. initiated edema and hyperkalemia after 8 days of treatment.

DISCUSSION

The initial recommended storage procedure for tetrahydrocannabinols included the use of amber bottles, a nitrogen atmosphere, and temperatures below 0° (4). Subsequent stability data indicated that 4° would suffice, and this change avoided cracking of bottles due to cannabinoid expansion at lower temperatures. Even at subzero temperatures, a surface layer of black material formed on Δ^8-tetrahydrocannabinol samples after several months of storage. Occasionally, this black material was found at deeper points in the sample, indicating that some chemical change had occurred during packaging. Dark surface material was rarely seen in Δ^8-tetrahydrocannabinol samples.

Various organic solvents solubilized cannabinoids to the extent obtained with ethanol but were not biologically useful. The addition of aqueous diluents to cannabinoid solutions in organic solvents resulted in suspensions with varying physical appearance of the flocculent. The final concentration of tetrahydrocannabinols was well below 0.5%, and the suspensions were adversely endowed with possible induction of embolism and the biological side effects of the organic solvent. The use of small quantities of emulsifier with large quantities of cannabinoid often led to suspensions upon introduction of an aqueous diluent. Viscous solvents like sesame oil, propylene glycol, and glycerol were modest solubilizers, but the usefulness of cannabinoid solutions with these solvents for biological trials was restricted by their viscosity. An extreme example of viscosity was seen in the formation of a paste between cannabinoid and polysorbate 80, which is a liquid. Although the results of the solubility study indicated that most of the solvents tested could be used for some biological trials (*e.g.*, single injections or properly spaced administrations to minimize vehicle toxicity), aqueous systems would be preferable for parenteral chronic treatments. The potential usefulness of a vehicle also depended upon its lack of hemolytic activity. Whereas the sesame oil–polysorbate 80 and sesame oil–polyvinylpyrrolidone caused no hemolysis during 28 days of intravenous administration to monkeys, sodium glycocholate evoked hemolysis within a few days.

Selection of a vehicle for administration of cannabinoids necessitated a compromise among various factors. These included the concentration of the cannabinoid, the concentration of the dispersant, the frequency of formulation, the physical stability of the injectable, the duration of treatment, and the specific sensitivity of a species to the dispersant. Because of the difficulty in manipulating cannabinoids, particularly in the preparation of aqueous systems

containing high concentrations of tetrahydrocannabinol, it is worthwhile to emphasize the time element involved in the preparation of a formulation. Whereas formulation time may not be critical in acute or subacute studies, it is a significant factor in chronic investigations where fresh, daily formulations may be required. For example, it took 5–15 min. to admix cannabinoid and polysorbate 80 plus 15–30 min. to triturate the admixture with isotonic saline to yield emulsions with 20–100 mg./ml. cannabinoid and concentrations of polysorbate 80 below 10%.

Formulations of cannabinoids were prepared in sesame oil at concentrations up to 400 mg./ml. which were suitable for chronic oral trials (90–120 days) in rats and monkeys (3, 5). Sesame oil has been an acceptable medicinal vehicle for many years, but a major side effect has been fecal softening (6). The use of concentrated cannabinoid solutions maintained a reasonable volume of sesame oil for each animal and reduced the laxative effect of the vehicle. Stock solutions of tetrahydrocannabinols in sesame oil were chemically stable for months[8]. Oral acute or subacute treatments with cannabinoid in low concentrations of ethanol or polysorbate 80 were tolerable. The sesame oil–polysorbate 80 emulsion should be useful orally because large volumes may be administered with relative safety.

The most successful injectable prepared at a reasonable concentration of cannabinoid (1–4%) was the sesame oil (10–13%)–polysorbate 80 (0.4–1.0%)–saline formulation. The preparation has been used intravenously with and without tetrahydrocannabinol for 28 consecutive days[9] and intermittently for several months in rhesus monkeys with minor effects ascribable to the vehicle (7). Emulsions could be prepared in a few minutes from stock solutions of cannabinoid in sesame oil. Such emulsions separated into two homogeneous phases, the upper containing most of the cannabinoid. However, because the cannabinoid in the upper layer remained dispersed in its phase, shaking restored the emulsion for a sufficient time interval to inject the material.

Other useful emulsions were comprised of sesame oil (10%) plus polyvinylpyrrolidone (4.5%), or sesame oil (10%) plus poloxalene (1%), and dispersed amounts of cannabinoid up to 1% for the polyvinylpyrrolidone system and 3% for the poloxalene emulsion. In preliminary intravenous trials, the polyvinylpyrrolidone emulsion was toxic in the dog but the poloxalene emulsion was not. Toxicity data on polyvinylpyrrolidone alone (8) and poloxalene alone (9) are available.

Stable emulsions of cannabinoid at relatively high concentrations were achieved in 5–10% polysorbate 80, but this vehicle was extremely toxic in the dog, similar to polysorbate 20 (10). Concentrated cannabinoid emulsions also required excessive preparation time, particularly if the concentration of polysorbate 80 was reduced below 10%. Physical stability of these emulsions only extended over a few days when globules formed and produced an oily surface film which would not redisperse.

It would seem helpful to review briefly the types of formulations reportedly used in marijuana studies. Of the 40 papers examined, 17% gave complete details of preparation, 75% simply mentioned the vehicle, and less than 30% provided information as to the concentration of cannabinoid achieved. Formulations used for oral administration included olive oil in the rat (11); propylene glycol in the mouse and rabbit (12); 5% gum acacia–saline containing EMUL-4767 in the mouse, cat, and squirrel monkey (13); 10% polysorbate 80–saline in the rat and mouse (14); and 95% ethanol plus cherry syrup or a soft drink in the human (2, 15).

Intraperitoneally, cannabinoids have been given in propylene glycol to the mouse and rabbit (12) and to the dog and monkey (16), in ethanol to the mouse and rat (17, 18), in polysorbate–saline to the mouse and rat (14, 19), in propylene glycol–polysorbate–saline to the rat (20, 21), in polyethyleneglycol 400–polysorbate–saline to the rat (22), in ethanol–polysorbate–saline to the rat (23), in polyethyleneglycol 300 to the rabbit and cat (24), in olive oil to the rat (25) and to the hamster and rabbit (26), in 5% gum arabic with traces of sesame oil or glycerin to monkeys (27), in peanut oil to the mouse and rat (28), and in 5% bovine serum albumin to the rat (29). Autoradiographic data have demonstrated that the intraperitoneal route for cannabinoid administration is not desirable (30).

Intravenous formulations included sesame oil–saline in the rat (11) and in the rabbit (31); propylene glycol in the mouse (12) and in the dog and monkey (16); polysorbate–saline in the mouse and rat (14) and in the cat (32, 33); plasma lipids in the rat (34); bovine serum albumin in the rat (35); homologous plasma in dogs (36); serum–propylene glycol in the rat, hamster, and rabbit (26); polyethyleneglycol 300 in the rabbit (24); 10% polyvinylpyrrolidone–saline in the rat (37); and Krebs–Henseleit bicarbonate in rats (38). A new technique exploited aspects of the normal lipid transport in rats (39).

Propylene glycol has been used subcutaneously in various species (16), as has olive oil–polysorbate (26). In the pigeon, 5% tyloxapol[10] has been used intramuscularly (40). Parenteral trials with 5.5% sodium glycocholate in the present study and 1% gelatin in a previous study were not fruitful[11].

CONCLUSIONS

The present investigation indicated that the use of concentrated solutions of cannabinoids in organic solvents (ethanol, dimethyl sulfoxide, propylene glycol, *etc.*) limited the volume of solvent being introduced into the animal to a relatively safe level. In acute or subacute trials, the influence of the vehicle may not have hampered interpretation of the pharmacological observations; but in chronic studies, vehicle toxicity could be manifest. Whereas homologous serum components may be useful dispersants of the cannabinoids, preparation of such formulations for chronic trials is impractical. With heterologous serum components or polysaccharide-like materials, repetitive administration could potentially exert adverse immunological responses in chronic trials.

Suspending agents like polysorbate offered useful aqueous systems, but species sensitivity to polysorbate presented a formidable difficulty. Emulsions with natural oils and emulsifiers afford a good approach to injectables for chronic studies. It seems clear now that the chemical stability of tetrahydrocannabinols is much greater than originally realized. Maintenance of stock solutions of cannabinoids in suitable solvents for the preparation of physically stable emulsions is a reality.

REFERENCES

(1) R. Mechoulam, *Science*, **168,** 1159(1970).

(2) L. E. Hollister, *ibid.*, **172,** 21(1971).

(3) G. R. Thompson, H. Rosenkrantz, and M. C. Braude, *Pharmacologist*, **13,** 296(1971).

(4) R. T. Turk, R. N. Phillips, J. E. Manno, N. C. Jain, and R. B. Forney, *Toxicol. Appl. Pharmacol.*, **17,** 310(1970).

(5) Y. K. Luthra, H. Rosenkrantz, N. L. Muhilly, G. R. Thompson, and M. C. Braude, *Abstr. 162nd National Meeting, Amer. Chem. Soc.*, **1971,** 212.

(6) "Pharmacological Basis of Therapeutics," 3rd ed., L. S. Goodman and A. Gilman, Eds., Macmillan, New York, N. Y., 1965.

(7) J. Martinez and U. Schaeppi, *Pharmacologist*, **13,** 246(1971).

(8) L. W. Burnette, *Proc. Sci. Sect. Toilet Goods Ass.*, **38,** 1 (1962).

(9) R. M. Nalbandian, A. Hymes, and R. Henry, *Bull. Pathol.*, **10,** 90(1969).

(10) L. S. Marks and S. N. Kolmen, *Amer. J. Physiol.*, **220,** 218 (1971).

(11) S. Agurell, I. M. Nilsson, A. Ohlsson, and F. Sanberg, *Biochem. Pharmacol.*, **18,** 1195(1969).

(12) H. I. Bicher and R. Mechoulam, *Arch. Int. Pharmacodyn. Ther.*, **172,** 24(1968).

(13) S. Irwin, Report to the Committee on Problems of Drug Dependence, *Nat. Acad. Sci. USA*, **1969,** 6142.

(14) R. N. Phillips, R. F. Turk, and R. B. Forney, *Proc. Soc. Exp. Biol. Med.*, **136,** 260(1971).

(15) H. Isbell, C. W. Gorodetzsky, D. R. Jasinski, U. Claussen, F. von Spulak, and F. Korte, *Psychopharmacologia*, **11,** 184(1967).

(16) Y. Grunfeld and H. Edery, *ibid.*, **14,** 200(1969).

(17) E. A. Carlini, *Pharmacology*, **1,** 135(1968).

[8] Communication obtained under National Institute of Mental Health contract elsewhere.

[9] Unpublished data.

[10] Triton X-100.

[11] These vehicles were tested in collaboration with Dr. Monroe E. Wall.

(18) R. K. Siegel and J. Poole, *Psychol. Rep.*, **25,** 704(1969).
(19) J. C. Garriott, L. J. King, R. B. Forney, and F. W. Hughes, *Life Sci.*, **6,** 2119(1967).
(20) R. K. Kubena and H. Barry, III, *J. Pharmacol. Exp. Ther.*, **173,** 94(1970).
(21) R. D. Sofia and H. Barry, III, *Eur. J. Pharmacol.*, **13,** 134 (1970).
(22) L. Maitre, M. Staehelin, and H. J. Bein, *Agents Actions*, **1,** 136(1970).
(23) O. A. Orsingher and S. Fulginiti, *Pharmacology*, **3,** 337 (1970).
(24) F. Lipparini, A. S. de Carolis, and V. G. Longo, *Physiol. Behav.*, **4,** 527(1969).
(25) C. J. Miras, "Hashish—Its Chemistry and Pharmacology," Little, Brown, Boston, Mass., 1965, p. 37.
(26) H. B. Pace, W. M. Davis, and L. A. Borgen, *Ann. N. Y. Acad. Sci.*, **191,** 123(1971).
(27) C. L. Scheckel, E. Boff, P. Dahlen, and T. Smart, *Science*, **160,** 1467(1968).
(28) F. J. A. Vieira, M. B. Agular, J. W. Alencar, A. P. Seabra, B. M. Tursch, and J. Lecleroq, *Psychopharmacologia*, **10,** 361(1967).
(29) W. L. Dewey, T. Peng, and L. D. Harris, *Eur. J. Pharmacol.*, **12,** 382(1970).
(30) B. T. Ho, G. E. Fritchie, L. F. Englert, W. M. McIsaac, and J. E. Idanpaan-Keikkila, *J. Pharm. Pharmacol.*, **23,** 309(1971).
(31) S. Agurell, I. M. Nilsson, A. Ohlsson, and F. Sanberg, *Biochem. Pharmacol.*, **19,** 1333(1970).
(32) E. S. Boyd and D. A. Meritt, *Arch. Int. Pharmacodyn. Ther.*, **153,** 1(1965).
(33) R. Dagirmanjian and E. S. Boyd, *J. Pharmacol. Exp. Ther.*, **135,** 25(1962).
(34) H. D. Christensen, R. I. Freudenthal, J. T. Godley, R. Rosenfeld, G. Boegli, L. Testino, D. R. Brine, C. G. Pitt, and M. E. Wall, *Science*, **172,** 165(1971).
(35) J. R. Milzoff, D. J. Brown, and C. J. Stone, *Fed. Proc.*, **30,** 1381(1971).
(36) C. A. M. Sampaio, A. J. Lapa, and J. R. Valle, *J. Pharm. Pharmacol.*, **19,** 552(1967).
(37) D. C. Fenimore and P. R. Loy, *ibid.*, **23,** 310(1971).
(38) B. R. Manno, J. E. Manno, G. S. Kilsheimer, and R. B. Forney, *Toxicol. Appl. Pharmacol.*, **16,** 79(1970).
(39) C. W. Christensen, J. B. Best, and R. A. Herin, *Fed. Proc.*, **30,** 1017(1971).
(40) D. E. McMillan, L. S. Harris, J. M. Frankenheim, and J. S. Kennedy, *Science*, **169,** 501(1970).

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Decomposition of Benzoic Acid Derivatives in Solid State

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Abstract □ A series of solid, substituted benzoic acids (p-XC_6H_4COOH), which decompose into a liquid (XC_6H_5) and carbon dioxide, were studied. For decomposition to take place below the melting point, the σ value must be less than -0.35; the decomposition then follows Bawn-type kinetics. Neither solid (k_s) nor liquid (k_l) decomposition constants show isokinetic relations at their melting points. However, log k_s is proportional to $1/T_m$, where T_m is the absolute melting temperature, much as was found for vitamin A esters.

Keyphrases □ Decomposition rates of solid compounds—p-substituted benzoic acid derivatives □ Solid-state decomposition—p-substituted benzoic acid derivatives □ Benzoic acid derivatives—solid-state decomposition, rate constants

Many studies have been conducted related to decomposition rates of solid compounds. A great majority of these have concerned inorganic salt decompositions [carbonates (1–9), oxalates (10–17), and permanganates (18–21)], and several general patterns have been proposed as primary mechanisms in the decompositions; most notable of these are the Prout–Tompkins model (21) and the power laws (22–25). Some studies have been reported in the pharmaceutical literature, notably the ones by Leeson and Mattocks (26), by Kornblum and Sciarrone (27), by Garrett *et al.* (28), and by Guillory and Higuchi (29).

Attempts to correlate decompositions in the solid state with usual substituent parameters in homologous series have not met with success. Dorko *et al.* (30) found no such correlation in a study of substituted tosylates, and Meyers *et al.* (31) found that in the reaction R′COONa + R″COOH → R′COOH + R″COONa, substituent σ values were the governing parameters in that σ' would have to be larger than σ'' for the reaction to occur; this, in essence, is paramount to the time-tested rule that "the stronger acid drives out the weaker acid." The latter two studies aimed at the importance of the chemical factors involved in reactivity in the solid state; whereas, in general, physical parameters (active sites, dislocations, *etc.*) have been the bases for proposed hypotheses.

The pharmaceutical literature has partially touched upon the importance of liquid layers as mediators of the actual decompositions (26, 29), whereas the remaining great majority of the cases cited dealt with reactions of the type solid → solid + gas. The work dealing with aspirin anhydride (28), as well as the work