Cannabis sativa L. (Marijuana) IV: Chemical Basis for Increased Potency Related to Novel Method of Preparation

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Abstract □ Recent reports from the drug subculture in this country indicate that the simultaneous use of marijuana teas together with smoking previously boiled marijuana plant material results in more profoundly experienced psychotropic effects. It was found that the boiling water treatment of marijuana removes water-soluble materials equivalent to 30% of the weight of the plant material, thus leading to marijuana correspondingly enriched in cannabinoids, including (−)-*trans*-Δ⁹-tetrahydrocannabinol, one of the major psychoactive compounds present in the plant. This finding explains, in part, the reputed increased pharmacological effects resulting from this newly described method of marijuana use. Moreover, the potential dangers inherent in the described method are discussed.

Keyphrases □ *Cannabis sativa*—new method of street use, chemical basis of increased potency, GLC □ Marijuana—new method of street use, chemical basis of increased potency, GLC □ Cannabinoids—stability following boiling water treatment, related to new street use to increase potency, GLC

Various preparations derived from the plant *Cannabis sativa* L. have long been used for their intoxicating properties (1). In the United States, the plant is usually employed as a euphoriant by means of smoking cigarettes or pipes containing marijuana[1] or marijuana admixed with other substances (2). It is generally accepted that (−)-*trans*-Δ⁹-tetrahydrocannabinol (I) is the major psychoactive constituent of marijuana (3). Marijuana is considered to be of high potency when the content of I is approximately 1% (w/w) (4). This report concerns a novel method of marijuana preparation and use among certain fringe groups of the drug subculture in this country[2].

The marijuana is first slurried with sufficient water to cover the plant material, and this mixture is subsequently boiled in a suitable container for 1 to several hours. Additional portions of fresh water are sometimes added periodically to maintain the level of the liquid and thus prevent possible charring of the marijuana. Upon termination of the boiling process, the hot mixture is allowed to cool to room temperature and is then strained through several layers of cheesecloth.

The resulting filtrate ("marijuana tea") is reserved and stored in a refrigerator because it has been found that the tea is prone to mold growth. The marijuana remaining on the filter is removed, manually expressed free of excess liquid, which is added to the reserved tea, and finally spread out and allowed to air dry. In some reported cases, the damp marijuana is dried using an ordinary baking oven set at moderate heat. The resulting dried marijuana plant material is subsequently formed into cigarettes in the usual manner, with no adjustment being made in the approximate weight of plant material used to prepare individual cigarettes. These cigarettes are smoked normally except for the following major variation. Just prior to smoking, the reserved marijuana tea is consumed at once. Those persons employing marijuana in this fashion claim that the subsequent effects of the drug are perceived to be significantly more profound, both in terms of intensity and duration, than are the effects experienced by smoking marijuana prepared by more conventional means.

The results of studies designed to assess the possible chemical consequences from this previously unreported utilization of marijuana are reported.

[1] The term marijuana as used here refers to the crushed leaves, stems, and flowering tops of *C. sativa*.

[2] It is presently not possible to determine accurately the extent of this practice. However, essentially similar practices have been related by particularly reliable sources from various areas of the eastern United states, including Massachusetts, Pennsylvania, New Jersey, and Delaware.

Table I—Effect of Prolonged Boiling Water Treatment on the I Content of Marijuana

Marijuana Material	I, % Calculated	I, % Found[a]
Untreated marijuana	1.86[b]	1.90
Boiling water-treated marijuana	2.65[c]	2.56

[a] The GLC peaks identified with I were further confirmed in separate experiments using combined GLC–mass spectrometry according to procedures described earlier (9). [b] From the supplier's assay results. [c] Calculated by assuming that a total of 1.86 g. of I remained unchanged in the 70 g. of dried, boiling water-treated marijuana derived from 100 g. of starting untreated marijuana material, as described under *Preparation of Boiling Water-Treated Marijuana and Marijuana Tea.*

EXPERIMENTAL

Plant Material and Preparation of Standard Solutions—Marijuana plant material and all authentic cannabinoids[3] were obtained from the National Institute of Mental Health. The coarsely milled and air-dried marijuana represented female plant material, designated as Campus Mexican Female, which had been grown for 14 weeks and subsequently harvested at the University of Mississippi during the 1971 season. The GLC tracings and other data accompanying this material indicated the following cannabinoid content: I, 1.86% [84% as the acid (V), which acid (A or B) not specified]; II, 0.03%; III, 0.19%; and IV, not quantitatable. Separate standard solutions of each authentic cannabinoid were prepared in 95% ethanol to furnish a final concentration of 0.5 mcg./μl. in each case. These solutions were used to obtain the standard tracings for the GLC analyses and were stored at −5° in the dark prior to use.

GLC—All analyses were carried out on a modified gas chromatograph[4] equipped with a hydrogen flame-ionization detector and a 6-mm. o.d. × 2-mm. i.d. × 1.83-m. glass column packed with 80–100 mesh Supelcoport, AW, DMCS coated with 3% OV-17[5]. The instrument was modified such that the exit end of the column led directly to the base of the flame tip while the other end of the column

[3] (−)-*trans*-Δ⁹-Tetrahydrocannabinol (I), (−)-*trans*-Δ⁸-tetrahydrocannabinol (II), (−)-*trans*-cannabidiol (III), and (−)-cannabinol (IV).

[4] Gow-Mac 69-750 FID, Gow-Mac Instrument Co., Madison, N. J.

[5] Supelco Inc., Supelco Park, Bellefonte, Pa.

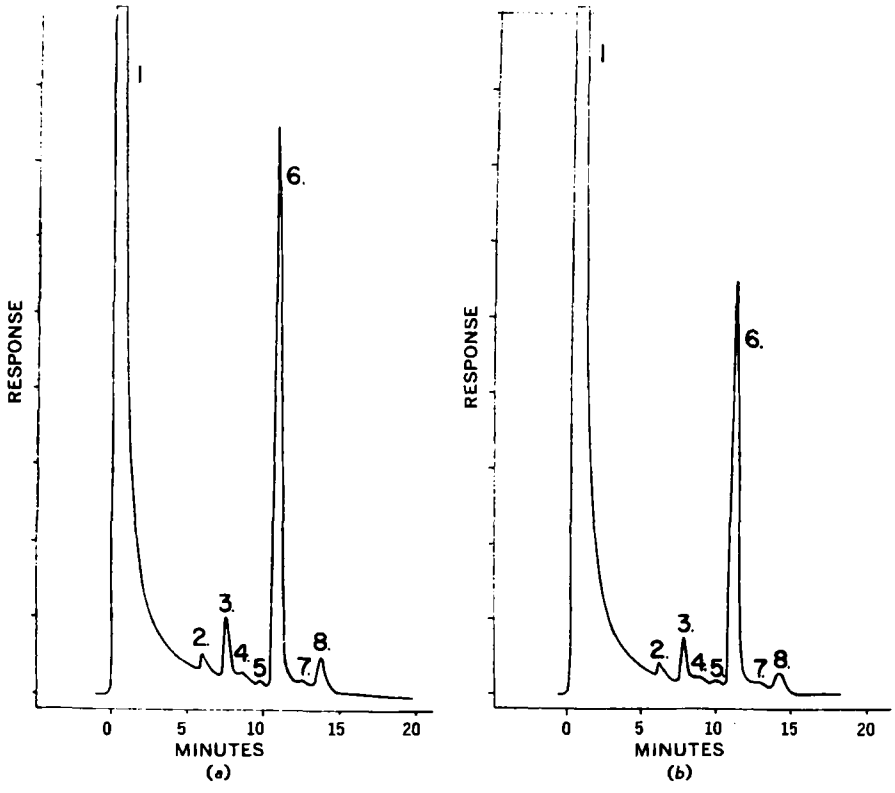

Figure 1—*Gas–liquid chromatograms of marijuana extracts (see text for method of preparation).* (a) *Extract A (boiling water-treated marijuana).* (b) *Extract B (untreated marijuana). Comparison of* a *and* b *reveals only quantitative differences. The analogous peaks (boiling water-treated marijuana* versus *untreated marijuana) are in the approximate ratio of 1.4:1, thus showing enrichment of the cannabinoids in the boiling water-treated marijuana. Key: 1, solvent; 2, unknown; 3,* (—)-trans-*cannabidiol; 4, unknown; 5,* (−)-trans-Δ^8-*tetrahydrocannabinol; 6,* (−)-trans-Δ^9-*tetrahydrocannabinol; 7, unknown; and 8,* (−)-*cannabinol.*

extended nearly to the injector port septum, thus resulting in an essentially all-glass system and allowing for on-column injection of the samples. The injector port and detector block temperatures were maintained at 263° while the column was operated isothermally at 203°. Attenuation was 7×10^{-11} amperes full scale for all analyses. Helium was used as the carrier gas at a flow rate of 10 ml./min.; hydrogen and dry air flow rates were 20 and 250 ml./min., respectively.

A strip chart recorder[6] with a chart speed of 0.508 cm./min. was employed. The peak areas recorded for all analyzed samples were calculated by the triangulation method (area = height × width at half-height) and compared with standard curves prepared by plotting the areas against known concentrations of standards analyzed in a similar manner. The results were found to be linear over the concentration ranges used. In those cases requiring derivatization (silylation), 0.5 ml. of the particular sample was evaporated using flowing nitrogen. The resulting dry residue (in a Teflon-lined screw-capped vial)[7] was treated with 0.5 ml. of a silylating reagent[8] and warmed (50°) for 3 min. It was found that derivatization was quantitative under these conditions, and subsequently an aliquot (usually 0.5–1.5 μl.) of the reaction mixture could be injected into the column.

Preparation of Boiling Water-Treated Marijuana and Marijuana Tea—A total of 100 g. of marijuana previously slurried with 1500 ml. of distilled water was continuously refluxed for 5 hr. using a Clevenger apparatus (for oils lighter than water) (5). No precautions were taken to exclude air or light from the boiling mixture. Following refluxing, the slurry less the volatile oil (0.2 ml.) was cooled to room temperature and suction filtered. The volume of the dark-brown filtrate was approximately 1300 ml. Finally, the plant material remaining on the filter was alternately washed and expressed, using small portions of hot water until the last wash filtrates were nearly colorless. These washings were combined with the initial filtrate until the total volume was adjusted to 1500 ml. This solution (marijuana tea) was frozen and lyophilized to give 30 g. of a dark-brown residue, which was stored under nitrogen at 4° for future chemical and pharmacological study. The remaining damp marijuana was allowed to air dry at ambient (23–25°) temperature for 5 days. This air-dried material was found to weigh 70 g. and was designated as boiling water-treated marijuana.

GLC of Boiling Water-Treated Marijuana and Untreated Marijuana—Fifty grams of boiling water-treated marijuana was percolated at a slow rate, using a total of 2000 ml. of 95% ethanol. The combined percolates were evaporated *in vacuo* to give a green syrup (about 25 ml.), which was brought to a volume of exactly 50 ml. with 95% ethanol. An aliquot (0.5 ml.) of this ethanolic solution was diluted using 95% ethanol to furnish 10 ml. of Extract A. Similarly, 50 g. of untreated marijuana was processed to furnish 10 ml. of Extract B. Aliquots (0.5–1.5 μl.) of Extracts A and B were analyzed by GLC to determine the cannabinoid profiles of the boiling water-treated marijuana and the untreated marijuana, respectively (Figs. 1*a* and 1*b*).

RESULTS AND DISCUSSION

It was found that 30% of the dry weight of the marijuana studied was removed by treatment with boiling water under conditions simulating those employed by certain members of the drug subculture. Thus, the loss of substantial quantities of water-soluble substances leads to marijuana (*viz.*, boiling water-treated marijuana) enriched in water-insoluble components, including cannabinoids such as I, a major psychoactive component of marijuana (Figs. 1*a* and 1*b*). For example, the boiling water-treated marijuana contained 1.4 times more I than was found in the untreated marijuana (Table I).

Under the conditions of these experiments, V was quantitatively decarboxylated to furnish the psychoactive I (Figs. 2*a* and 2*b*). This was not unexpected since it had earlier been pointed out that the cannabinoid acids rapidly decarboxylate at temperatures approaching 103° (6). What was surprising, but not totally unexpected, was that the cannabinoids present in the original untreated marijuana were stable when subjected to the relatively drastic conditions of prolonged boiling. Indeed, when the GLC analyses of the boiling water-treated marijuana and the untreated marijuana are compared (Figs. 1*a* and 1*b*), it is clear that the differences noted are quantitative and not qualitative. It was recently shown (7, 8) that certain cannabinoids are especially stable when they are present in crude preparations derived from *C. sativa*. In these cases, the complex

[6] Model MP-1027/1 mv., having a full-scale span of 1 mv.; McKee-Pedersen Instruments, Danville, Calif.
[7] Reacti-vial, Pierce Chemical Co., Rockford, Ill.
[8] TRISIL/BSA, *N,O*-bis(trimethylsilyl)acetamide in pyridine, Pierce Chemical Co., Rockford, Ill.

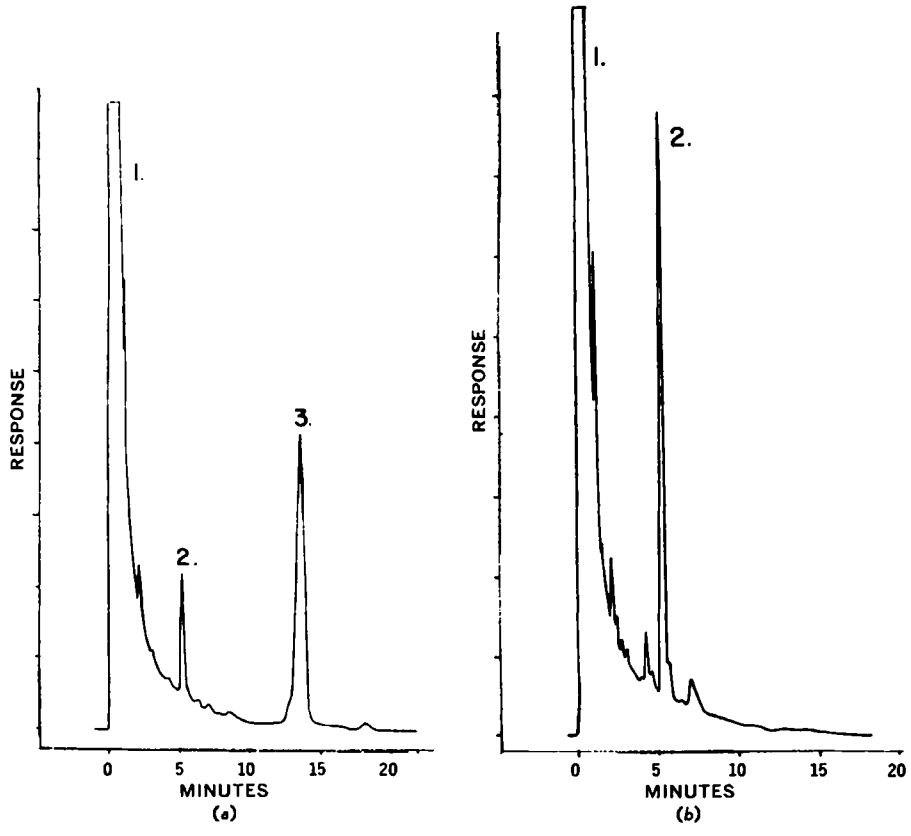

Figure 2—*Gas–liquid chromatograms of silylated marijuana extracts (see text for method of preparation). (a) Extract B (untreated marijuana) showing total (−)-trans-Δ⁹-tetrahydrocannabinol content was 84% (−)-trans-Δ⁹-tetrahydrocannabinol acid and 16% (−)-trans-Δ⁹-tetrahydrocannabinol. (b) Extract A (boiling water-treated marijuana). The absence of the (−)-trans-Δ⁹-tetrahydrocannabinol acid – trimethylsilyl derivative peak and the presence of the (−)-trans-Δ⁹-tetrahydrocannabinol–trimethylsilyl derivative peak indicate that the (−)-trans-Δ⁹-tetrahydrocannabinol acid was quantitatively decarboxylated to furnish (−)-trans-Δ⁹-tetrahydrocannabinol as a result of the boiling water treatment. Key: 1, solvent; 2, (−)-trans-Δ⁹-tetrahydrocannabinol–trimethylsilyl derivative; and 3, (−) - trans - Δ⁹ - tetrahydrocannabinol acid–trimethylsilyl derivative.*

mixture of phytoconstituents apparently retards the decomposition of the cannabinoids. This phenomenon may also explain the stability of the cannabinoids remaining in the boiled crude marijuana plant material[9].

Hence, the reputed claims made for the increased biological potency of boiling water-treated marijuana appear to be related, at least in part, to increased concentrations of presently known psychoactive components. Obviously, individuals who smoke the same weight of this material as the untreated marijuana (as indeed they do, as mentioned earlier) would experience increased drug effects.

The biological activity of the aqueous extract (marijuana tea) taken concomitantly with the boiling water-treated marijuana has not yet been fully evaluated. Both chemical and pharmacological studies of the marijuana tea are currently underway in these laboratories and results will be reported subsequently.

Nevertheless, immediate attention is called to the possible serious consequences that may result from the oral self-administration of marijuana teas for the following reasons. Since drugs are commonly misrepresented in the illicit market, it is not unusual to find contraband marijuana admixed with various noncannabis substances (2). Consider, for example, a street sample of marijuana containing equal amounts of tobacco and marijuana. In this particular case, a tea prepared from this material would probably contain relatively large amounts of the poisonous alkaloid nicotine; under normal smoking conditions most of the nicotine content of tobacco is destroyed. Similarly, analysis of confiscated marijuana (10) showed it to be impregnated with significant amounts of lysergic acid diethylamide (VI). Although VI is not biologically active when smoked, dangerous effects could result from ingesting teas prepared from VI-containing marijuana. Moreover, it may not be unreasonable to assume that marijuana may be adulterated with other drugs which are not biologically active when smoked but which may induce a variety of undesirable physiological effects when ingested in teas.

[9] Moreover, in separate experiments, the boiling water-treated marijuana was shown to be biologically active (dog ataxia test) according to procedures described elsewhere (7).

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