ABSTRACT

Relatively little is known regarding the drug effects of marihuana in man, vis-à-vis the different methods of preparing the plant material prior to actual use. A search of the scientific literature reveals cryptic references which lead one to believe that there may indeed be subtle as well as frank pharmacological differences in the marihuana induced effects in humans. These differences may depend on the sundry methods whereby various hemp products are prepared and used throughout the world.

Although marihuana is usually employed in this country by means of smoking the crushed and dried plant material, recent reports related to us from certain fringe groups of the-drug subculture describe a heretofore unknown and novel method of marihuana preparation and use. Reputedly, the simultaneous ingestion of marihuana teas together with smoking cigarettes prepared from previously water-boiled marihuana plant material, results in increased psychotropic effects both in terms of intensity as well as duration. The known aspects of this ritual will be briefly described.

In our laboratories, using experimental conditions chosen to simulate the extemporaneous manner of preparation, it was found that the boiling water treatment of marihuana led to marihuana that was significantly enriched in cannabinoid substances, including delta-9-tetrahydrocannabinol which is considered to be the major psychoactive component of marihuana. This may explain, at least partly, the alleged claims made for the increased biological potency of marihuana so prepared. Furthermore, preliminary animal experiments indicate that marihuana teas may indeed exhibit biological activities. Finally, the potential health hazards inherent in this newly described practice, based on the fact that illicit marihuana is frequently misrepresented and adulterated with other drugs, will be discussed.

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INTRODUCTION

The Indian hemp plant, botanically known as Cannabis saliva L., has long been known to man as a source of numerous preparations possessing intoxicating properties (Bouquet 1950). It is presently debatable as to whether or not Cannabis is a monotypic genus (viz., represented by the single species sativa). Schultes (1970a) and Quimby et al. (1973) have dealt with this subject in some detail. For the purpose of the present discussion, hemp will be considered to be synonymous with Cannabis sativa L. The plant itself, probably native to central Asia, is now distributed throughout most temperate and tropical regions of the world where it is either cultivated or grows wild as an herbaceous annual weed. Cannabis is usually dioecious, that is, having the female and male flowers borne on separate plants. Although it was previously believed that the male plants were biologically inactive in terms of intoxicating properties, recent evidence has demonstrated that both female and male plants contain psychoactive constituents (Fetterman et al. 1971; Small and Beckstead 1973).

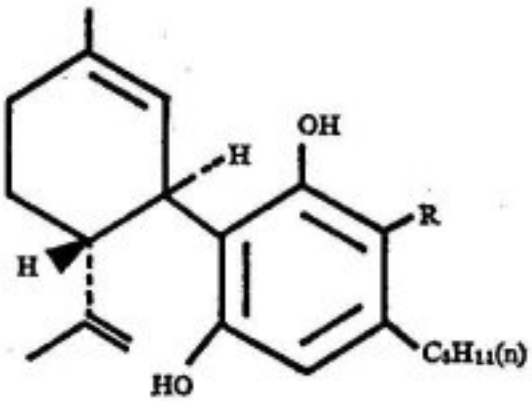
Hashish of the Middle East and charas or churrus of the Indian peninsula represent the resinous secretion that spontaneously exudes-from especially the flowering tops of the female plants. Ganja of India is prepared by collecting the tops of the female hemp plants; bhang refers to the product of the entire hemp plant or various mixtures of the leaves, stems and flowering tops. Marihuana, the product most often encountered in North America, is similar to bhang. Hashish is generally considered the most potent in terms of intoxicating properties, followed by ganja and bhang in order of decreasing potency. Nearly all these hemp preparations may be used alone or incorporated into various commodities suitable for eating, drinking, smoking and even snuffing (Bouquet 1950; 1951).

CHEMISTRY AND PHARMACOLOGY

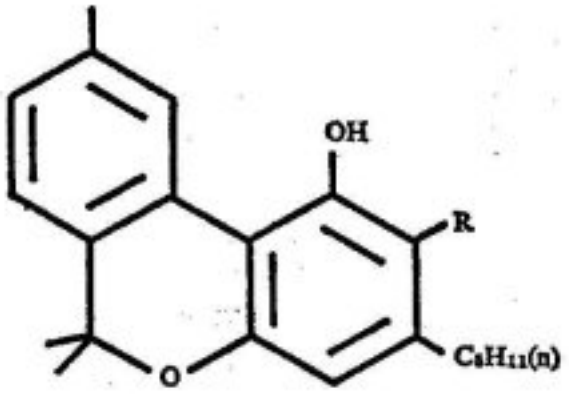
The chemistry and pharmacology of cannabis have been extensively reviewed and need not be discussed in detail here (Wolstenholme and Knight 1965; Mechoulam and Gaoni 1967; Joyce and Curry 1970; Gershon 1970; Mechoulam 1970a; Braude et al. 1971; Hollister 1971; Neumeyer and Shagoury 1971; U. S. DHEW 1971; Cotton 1971; Singer 1971; Mills and Brawley 1972; Nahas 1973; and U. S. DHEW 1972). Nevertheless, it will be useful to introduce certain relevant chemical and pharmacological data. From a chemical point of view, cannabis is unique in containing a large number of different but closely related fatsoluble compounds collectively referred to as cannabinoids (Figure 1) (Mechoulam and Gaoni 1967), that have thus far not been found elsewhere in nature. The majority of the cannabinoids have been isolated and identified only since 1964, when Gaoni and Mechoulam (1964) characterized the compound (—) trans-A9-tetrahydrocannabinoll (L9-THC) and identified it as the major

psychoactive constituent of hashish.

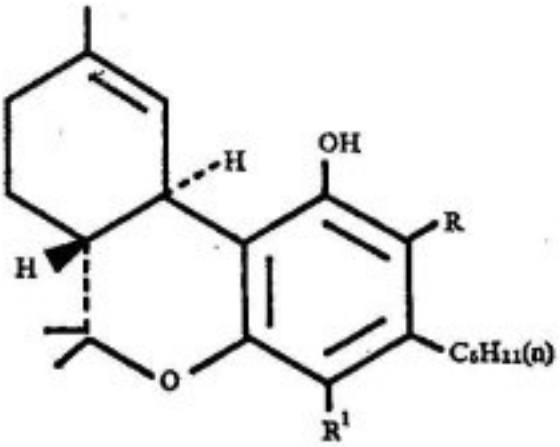
Figure 1. Some selected cannabinoids



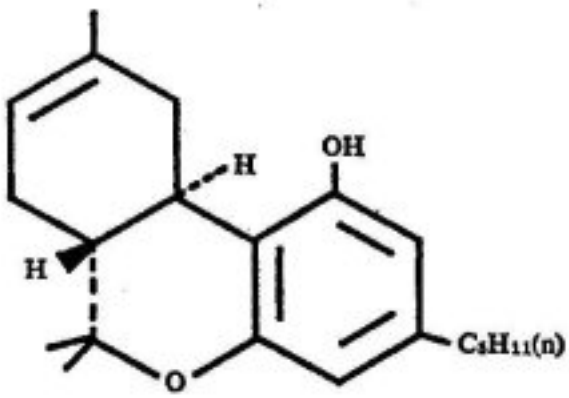
I, R=H, Cannabidiol (CBD)
II, R=COOH, Cannabidiolic Acid (CBDA)



III, R=H, Cannabinol (CBN)
IV, R=COOH, Cannabinolic Acid (CBNA)



V, $R=R^1=H$, Δ^9-Tetrahydrocannabinol (Δ^9-THC)
VI, R=COOH, $R^1=H$, Δ^9-Tetrahydrocannabinolic Acid A
VII, R=H, $R^1=COOH$, Δ^9-Tetrahydrocannabinolic Acid B



VIII, Δ^8-Tetrahydrocannabinol (Δ^8-THC)

Note: The Δ^9-THC acids are not biologically active *per se*, but are converted (decarboxylated) by heating (smoking) to furnish the psychoactive Δ^9-THC.

It is now generally accepted that 6,9-THC is one of the principal cannabinoid constituents and the major psychoactive component present in most hemp preparations (Isbell et al. 1967; Mechoulam et al. 1970a). A second minor cannabinoid, (-)-trans- As-tetrahydrocannabinol (A8-THC), has also been found to exhibit psychoactive properties (Grunfeld and Edery 1969; Hively et al. 1966) while the remaining cannabinoids have been shown to be lacking in observable psychopharmacological effects (Edery et al. 1971). Large quantities of both A9-THC and A8-THC have been synthesized and are available for research purposes. This is also the case for cannabis plant material and "crude" extracts therefrom (Scigliano and Waller 1970). However, pharmacological experiments in animals and humans have been carried out using 6,9- THC (Joyce and Curry 1970; Gershon 1970; Mechoulam 1970a; Braude et al. 1971; Hollister 1971; Neumeyer and Shagoury 1971; U. S. DHEW 1971; Cotten 1971; Singer 1971; Mills and Brawley 1972; Nahas 1973; and U.S. DHEW 1972).

It has been demonstrated that approximately 50 percent of the A9- THC content of marihuana cigarettes was delivered unchanged via the smoke to the lungs of the users, providing that the entire cigarettes, including the butts, were smoked (Manno et al. 1970). The 6,9-THC is apparently rapidly absorbed from the lungs and subsequently produces typical pharmacological effects. Although it has been reported that the ratios of the cannabinoids found in marihuana smoke approximate the ratios of the natural cannabinoids in the plant (Truitt 1971), relatively little is known concerning the chemistry and pharmacology of the smoke from cannabis preparations (Fentiman et al. 1973). Might there perhaps be present one or more components of the smoke which arc biologically active per se or which in some other way modify the biological effects of the psychoactive A9-THC?

Isbell and co-workers have reported that the absorption of A9-THC is approximately three times less effective by mouth than when smoked (Isbell et al. 1967).2 When considering the variety of hemp preparations containing cannabis or hashish which are intended to be taken by mouth, the question arises: are there present in these preparations heretofore undiscovered biologically active substances which could contribute to the overall perceived effects in humans? This important question has not been resolved to date.

In 1843 O'Shaughnessy described the preparation of the hemp confection known as majoon, "Four ounces of sidhee [Mang] and an equal quantity of ghee (clarified butter) are placed in an earthen or well-tinned vessel, a pint of water added, and the whole warmed over a charcoal fire. The mixture is constantly stirred until the water all boils away... the mixture is then removed from the fire, squeezed through cloth while hot — by which an oleaginous solution of the active principals and coloring matter of the hemp is obtained — and the leaves, fibres, etc., remaining on the cloth are thrown away. The green oily solution soon concretes into a buttery mass, and is then well washed by the hand with soft water so long as the water becomes colored. The coloring matter and an extractive substance are thus removed, and a very pale green mass of

the consistency of simple ointment remains. The washings are thrown away; Ameer [the proprietor of a place for hemp users in Calcutta] says that these washings are intoxicating and produce constriction of the throat, great pain and very disagreeable and dangerous symptoms [italics ours]." (344-345) Is this perhaps one of the earliest indications that cannabis contains water-soluble biologically active substances? We shall return to this interesting concept later. Incidentally, the majoon described above appears to be similar to the arabic confection dawamesc which the French physician Jacques-Joseph Moreau used to study the effects of hemp in humans (Nahas 1973).

Schultes' remarks concerning peyote cactus intoxication could apply as well to hemp: "The very real — and often overlooked — differences between peyote intoxication and mescaline intoxication must be constantly borne in mind. Amongst aboriginal users, it is the dried head of the cactus, with its total [italics ours] alkaloid content, that is ingested; mescaline injected is employed only experimentally and then produces the effects of one of the alkaloids without the physiological interaction of the others that are present in the crude plant material. As a consequence, descriptions of the visual hallucinations found in psychological writings should not necessarily be too closely evaluated with the visual effects experienced by Indian peyotists" (Schultes 1970b: 33). The points that we are trying to emphasize are (i) the Δ9-THC content of a particular cannabis preparation may not be a reliable index of psychoactive activity, (ii) possible pharmacological interactions between different hemp constituents, perhaps leading to subtle differences in perceived effects, cannot be and indeed should not be excluded at the present time.

STABILITY AND VARIATIONS IN POTENCY •

In 1970, during the course of a program designed to assess the chemical constituents of hemp preparations, we obtained for study a 43-year-old sample of an alcoholic cannabis fluidextract (Kubena et al. 1972). This preparation had formerly been official in the United States Pharmacopoeia (Pharmacopoeia 1926). Using gas-liquid chromatography, it was shown that the fluidextract contained 0.4% .6,9-THC, 0.1% cannabidiol and 0.04% cannabinol. Furthermore, we found that this preparation, even after the extended storage period of 43 years, still contained sufficient biological activity to induce the characteristic ataxia in dogs, following oral administration of the fluidextract to animals according to USP X (Ibid.).

These observations were totally unexpected because it had generally been assumed that cannabis preparations rapidly lost their potency with time (Bradbury 1899; Hamilton 1915; Eckler and Miller 1917; and Hamilton 1918). For example, Hooper (1894: 49) reported, "Ganjas always lose their strength when kept for some time, and many dealers in India obtain new

supplies annually, and always consider the drug worthless after being kept three years." The Dispensatory later stated, "It is recognized in India that ganja rapidly deteriorates on keeping, that which is one year old being not more than one-quarter as potent as the fresh drug, while two-year-old ganja is practically inert and is required by the Indian government to be burned in the presence of excise officers." (Wood et al. 1926 :278).

These conclusions have, in fact, extended to contemporary times (Trease and Evans 1966; Wallis 1967). But experimental data of greater significance resulted from further biological testing of the fluidextract. After the alcohol had been removed from the fluidextract, the residue was reconstituted in an inert vehicle, propylene glycol. Aliquots of the reconstituted mixture, calibrated to contain various amounts of z9-THC, were tested in an operant conditioning lever-pressing procedure, based on alternative responses of food approach and shock avoidance (Kubena and Barry 1972).

In this procedure, rats were trained to make one response following injection of A9-THC (4mg/Kg) and the alternative response after injection of the vehicle alone. Previous experiments with various drugs, including cannabinol, cannabidiol, morphine, cocaine, atropine, scopolamine, ethyl alcohol, chlorpromazine, chlordiazepoxide, pentobarbital, mescaline, LSD-25 and dimethyltryptamine showed that this bioassay was highly specific to A9-THC. Hence, this procedure can serve to furnish valuable information regarding specific measurements of the subjective states induced by 6,9-THC. Accordingly, psychoactive drugs can be shown to be similar or different, presumably on the basis of the induced perceptual states (Kubena and Barry 1969).

Table 1. The effect of prolonged boiling water treatment on the Δ^9-THC content of marihuana

Marihuana material	Δ^9-THC, Percent	
	Calculated	Found
Untreated marihuana	1.86[a]	1.90
Boiling water treated marihuana	2.65[b]	2.56

[a] Determined from the supplier's assay results.
[b] Calculated by assuming that a total of 1.86g of Δ^9-THC remained unchanged in the 70g of dried, boiling water treated marihuana derived from 100 g of starting untreated marihuana plant material.

The data in Table 1 shows the results of this bioassay and clearly indicates that the marihuana fluidextract was approximately three times more potent when compared with equivalent amounts of pure 69-THC. Unfortunately, the small amount of fluidextract available at the time precluded further studies to discover the phytoconstituents responsible for the observed increase in biological activity.

Presently there is an ever-increasing body of evidence which points to the fact that there may be heretofore uncharacterized biologically active constituents present in hemp. For example, contrary to previously published data (Persaud and Ellington 1967, 1968; Geber and Schramm 1969a, 1969b) which showed teratogenic effects in animals following injections of crude marihuana extracts, Borgen and co-workers (1971) were unable to reproduce these undesirable effects in rats, using pure [19-THC. These investigators concluded, "It is quite possible that A9-THC is indeed not teratogenic in the rat, but instead, another substance in the plant which occurred in the extracts was responsible for the defects observed previously. This other substance may be another of the cannabinoids known to exist in marihuana, or some other as yet unidentified compound" (485).

In a separate study, Karniol and Carlini (1972) found that the results from testing two different marihuana extracts indicated that they were approximately three to five times more active in rabbits, rats and mice when compared with the results obtained with 6,9-THC. Very recently, Galanter et al. (1973) reported that marihuana, calibrated to contain known doses of A9-THC, and pure 19-THC were not absolutely equivalent when smoked. In another investigation, an in vivo study (Poddar and Ghosh 1972) was made of the comparative effects following the administration of a cannabis extract and 69-THC on the activities of two rat liver enzymes, tryptophan pyrrolase and tyrosineac -ketoglutarate. Both the cannabis extract and the 6,9-THC increased the two enzyme activities, but it was found that the cannabis extract elicited a greater response than did equivalent amounts of A9-THC. In preliminary experiments Gill and collaborators (1970) presented evidence for the presence in cannabis of water soluble substances having pharmacological activities. It has also been reported (Klein et al. 1971) that a semi-purified alkaloid fraction derived from Cannabis sativa showed pharmacological activity (viz.— decreased motor activity) in mice.

The significance of the above examples as applied to humans, in terms of biological activities other than those attributed to A9-THC alone, remains to be established.

NOVEL METHODS OF USE

We have recently received reports (Segelman and Sofia 1973) indicating that certain fringe groups of the drug subculture in this country, are preparing and using marihuana according to a novel procedure. Briefly described, these individuals prepare and utilize marihuana as follows: the marihuana is first mixed with enough water to completely cover the plant material and is subsequently boiled for periods of time ranging from one to several hours. Additional amounts of fresh water are added from time to time in order to keep the plant material covered with liquid and thus prevent possible charring. The mixture is then allowed to cool and is passed through a cheesecloth filter. The filtrate (viz.—marihuana tea) is set aside and stored in a refrigerator because it has been found that the tea is prone to mold growth when kept at ambient temperatures. The boiled marihuana material remaining on the filter is removed, manually expressed free of entrapped liquid which is added to the reserved tea and finally spread out and allowed to spontaneously air-dry.

In some instances the damp marihuana is dried in household ovens, while certain more enterprising groups use hot-air hair dryers. The resulting dried marihuana is used to prepare cigarettes in the usual manner, with no adjustment being made in the approximate weight of plant material for individual cigarettes. These cigarettes are smoked normally except for the following variation: just prior to smoking, the reserved marihauna tea is consumed at once. We have presently no data regarding the amounts of tea ingested. Individuals employing the described procedure claim that the subsequent effects of the smoked marihuana are perceived to be significantly more profound, both in terms of intensity and duration, than are the effects experienced by smoking marihuana according to more conventional methods. We have not determined with certainty to what extent the described practice is carried out. However, essentially similar practices have been related by reliable sources from certain areas of the eastern United States, including New Jersey, Delaware, Pennsylvania, and Massachusetts.

We decided it would be worthwhile to study this problem under controlled laboratory conditions for the following reasons: first, it was of interest to assess the stability of the cannabinoids under the conditions of prolonged boiling. Reasonable stability of the compound Δ9-THC could only be assumed on the basis of the long history of hemp products taken in the form of infusions or confections of various kinds involving heating during some stage of their preparation (Bouquet 1950, 1951). Of course, as mentioned earlier, it was known that approximately 50 percent of the cannabinoids survived the heat of combustion generated during smoking (Manno et al. 1970), but no stability studies had been done on the cannabinoids under boiling water conditions. Second, it was important to determine whether or not the marihuana tea showed pharmacological activity.

In order to stimulate the boiling water treatment of marihuana as described above, the following

procedure was employed: a total of 100 g of marihuana [analyzed for Δ9-THC, 1.86% (84% as the acid); Δ8-THC, 0.03%; CTD, 0.19%; CBN, not quantitable] previously slurried with 1500 ml of distilled water was continuously refluxed for five hours using a Clevenger apparatus designed to collect volatile oils lighter than water. No precautions were taken to exclude light or air. Following refluxing, the slurry less the volatile oil was allowed to cool to room temperature and was suction filtered. The marihuana on the filter was washed with hot water and the wash filtrates were combined with the initial filtrate. This combined solution (viz.- marihuana tea) was frozen and lyophilized to give 30 g of a dark brown residue that was set aside for pharmacological testing. The remaining damp marihuana was allowed to air-dry for five days and was found to weigh 70 g. Thus, 30 percent of the dry weight of the original marihuana was removed by the boiling water treatment. The cannabinoid profiles of the boiling water treated marihuana and the untreated (non-boiled) marihuana were determined by gas-liquid chromatography (Figure 2a and 2b; see pages 282-285).

Table 2. Responses[a] of rats to treatment with Δ^9-THC compared with the 43-year-old marihuana fluidextract

Δ^9-THC mg/Kg	Δ^9-THC	Marihuana fluidextract (0.4% Δ^9-THC)
16.0	100	–
4.0	93	–
2.0	77	–
1.0	33	91
0.5	0	45
0.25	–	9
0	8	–
ED_{50} (mg/Kg)	1.40	0.51
95% Confidence limits	0.93–2.12	0.32–0.80

[a] Percentage Δ^9-THC response (approach for half the animals, avoidance for the others) in tests with several doses and the 43-year-old marihuana fluidextract containing this compound by rats trained to make differential responses to 4 mg/Kg and the non-drug control conditions (0mg/Kg). See Kubena and Barry (1972).

The data in Table 2 shows that the boiling water treated marihuana contained 1.4 times more Δ,9-THC than was found in the untreated marihuana. Under the conditions of the experiment,

the biologically inactive Δ,9-THC-acid was quantitatively decarboxylated to furnish the psychoactive Δ9-THC (Figure 3a and 3b). This was not unexpected since it was known (Mechoulam 1970b) that the cannabinoid acids smoothly decarboxylate at temperatures approaching 103°. The finding that was surprising was that the cannabinoids present in the original untreated marihuana proved to be stable when subjected to the relatively drastic conditions of prolonged boiling. In fact, if one compares the gas-liquid chromatography analyses of the boiling water treated marihuana and the untreated marihuana (Figure 2a and 2b), it is quite clear that they differ quantitatively and not qualitatively. We (Kubena et al. 1972) and others (De Zeeuw et al. 1972) have shown that many of the cannabinoids appear to be relatively stable when they are present in crude cannabis preparations. In such cases, the complex mixture of phytoconstituents apparently retards the decomposition of the cannabinoids, and this phenomenon may also explain the stability of the cannabinoids remaining in the boiled marihuana plant material.

Thus, the reputed claims made for the increased biological potency of the boiling water treated marihuana can be explained, in part, as follows: the boiling water treatment of marihuana removes water soluble materials equivalent to 30 percent of the weight of the starting plant material, thus leading to marihuana correspondingly enriched in water-insoluble compounds, including A.9-THC, one of the major psychoactive compounds present in the plant. Obviously, those persons who smoke the same approximate weight of this boiled material as the untreated marihuana, would experience more profound drug effects.

Table 3. The effect of Δ^9-THC and marihuana tea on hexobarbital sleeping time in mice[a]

Test compound	I.P dose mg/kg	N	Sleeping time in minutes (Mean ± S.E.)	Percent increase
Propylene glycol vehicle	–	16	49 ± 4	–
Δ^9-THC	5.0	8	49 ± 2	0
	10.0	8	100 ± 7[b]	104
	20.0	8	105 ± 12[b]	114
Distilled H_2O vehicle	–	16	41 ± 2	–
Marihuana tea	12.5	8	44 ± 4	7
	25.0	8	58 ± 5[b]	41
	50.0	8	59 ± 3[b]	44
	100.0	8	81 ± 8[b]	98

[a] Thirty minutes following administration of the test drugs or their vehicle each mouse was given an injection of hexobarbital sodium (125 mg/kg, I.P.). Sleeping time for each animal was measured by the time in minutes from the loss to regaining of the righting reflex observed for at least ten seconds after the animal was placed on its back.
[b] $p \leq 0.05$ when compared with its respective vehicle-treated control group.

Table 4. The effect of Δ^9-THC and marihuana tea in the hot plate analgesic test[a] in mice

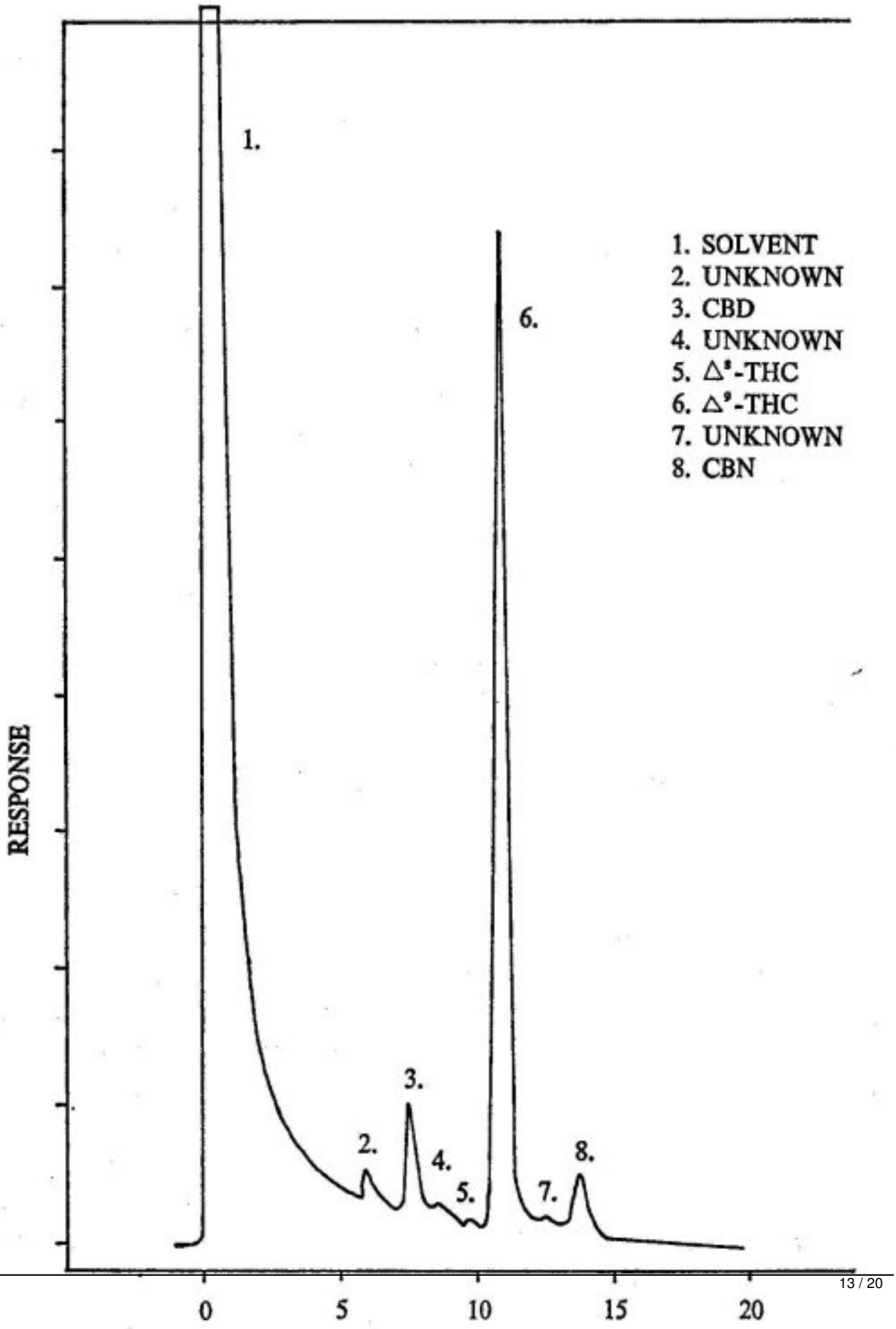
Test compound	I.P. doses mg/kg	Control reaction time[b]	Drug reaction time[b]	% Increase in mean reaction time	# *Analgesic*[c] # Tested
Propylene glycol vehicle	–	7.8 ± 0.7	7.7 ± 0.6	0.0	0/8
	0.3	8.3 ± 0.6	10.0 ± 1.5	20.7	3/8
	0.625	7.4 ± 0.5	11.6 ± 1.5[d]	57.3	4/8
Δ^9-THC	1.25	7.6 ± 0.4	11.0 ± 1.0[d]	45.2	5/8
	2.5	7.5 ± 0.6	11.7 ± 1.1[d]	56.2	5/8
	5.0	7.6 ± 0.7	12.7 ± 2.1[d]	68.7	5/8
	10.0	7.4 ± 0.6	16.8 ± 1.1[d]	126.2	7/8
Distilled water vehicle	–	7.9 ± 0.2	7.9 ± 0.5	0.0	0/8
	100.0	8.1 ± 0.7	8.9 ± 0.9	11.0	2/8
Marihuana	200.0	7.0 ± 0.5	9.6 ± 0.5[d]	37.0	4/8
tea	300.0	8.0 ± 0.6	12.4 ± 1.7[d]	54.7	6/8
	400.0	7.5 ± 0.6	12.0 ± 1.0[d]	60.6	7/8

[a] This method for assessing analgesic activity was based on the reaction time of mice to lick their forepaws and/or jump after exposure to a copper surface hot plate heated and maintained at 54 to 56°C. A control reaction time (measured to the nearest 0.1 second) was obtained 24 hours prior to any test for drug effect. Only those mice with a control reaction time of ten seconds or less were used. On the test day, mice were administered the test drugs or their vehicles and thirty minutes later each mouse in a group re-exposed to the hot plate surface and the reaction time once again recorded.

[b] These data represent the mean ± S. E. for 8 mice in a group.

[c] The number of mice per group showing a 40% or greater increase in their pre-drug reaction time. These data are used to calculate the ED_{50} value (95% confidence limits); for Δ^9-THC, 1.3 (0.5–3.1) and the marihuana tea 216.0 (135.0–346.0).

[d] $p \leq 0.05$ when compared with its respective pre-drug control reaction time.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3. CBD
4. UNKNOWN
5. Δ⁸-THC
6. Δ⁹-THC
7. UNKNOWN
8. CBN
RESPONSE
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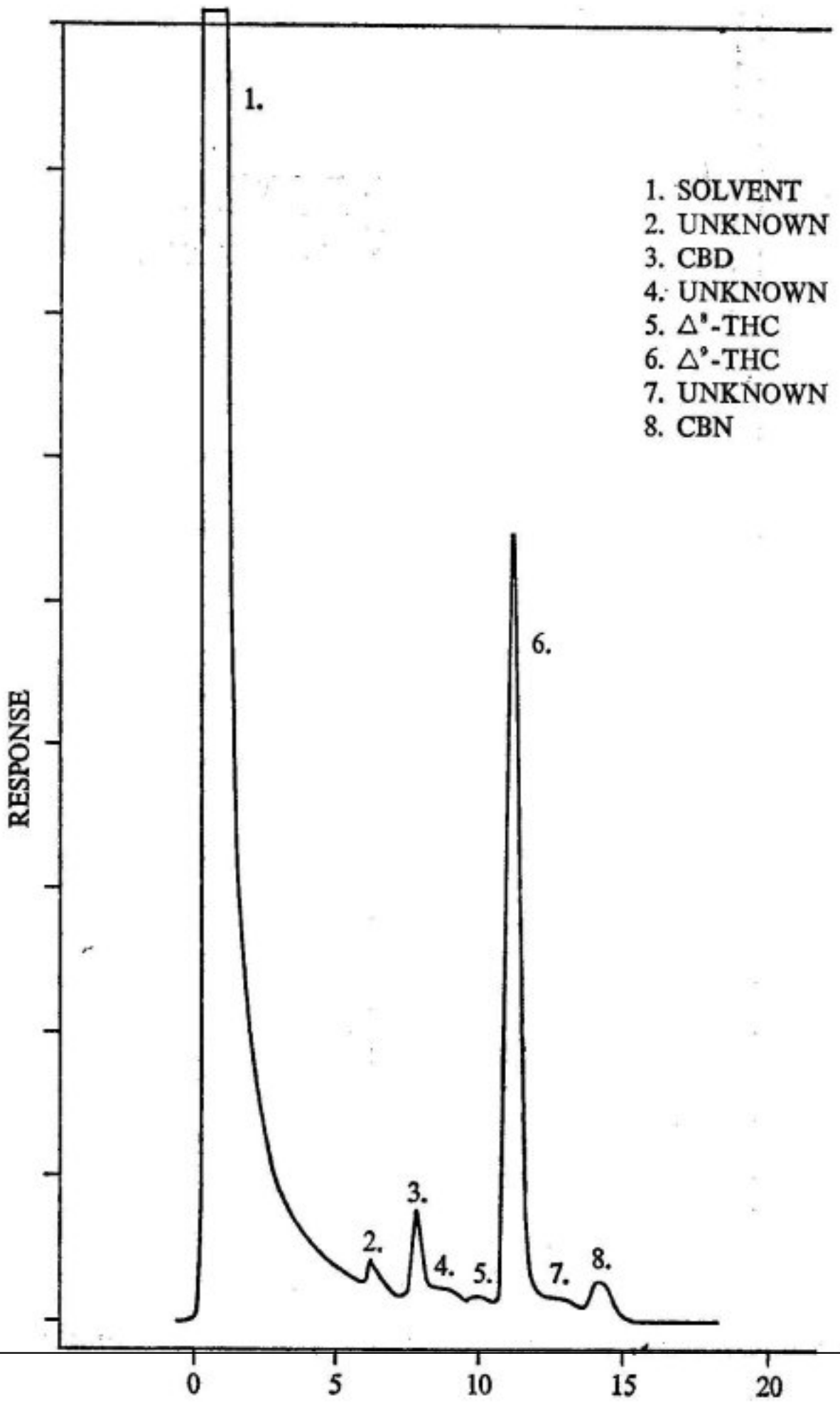
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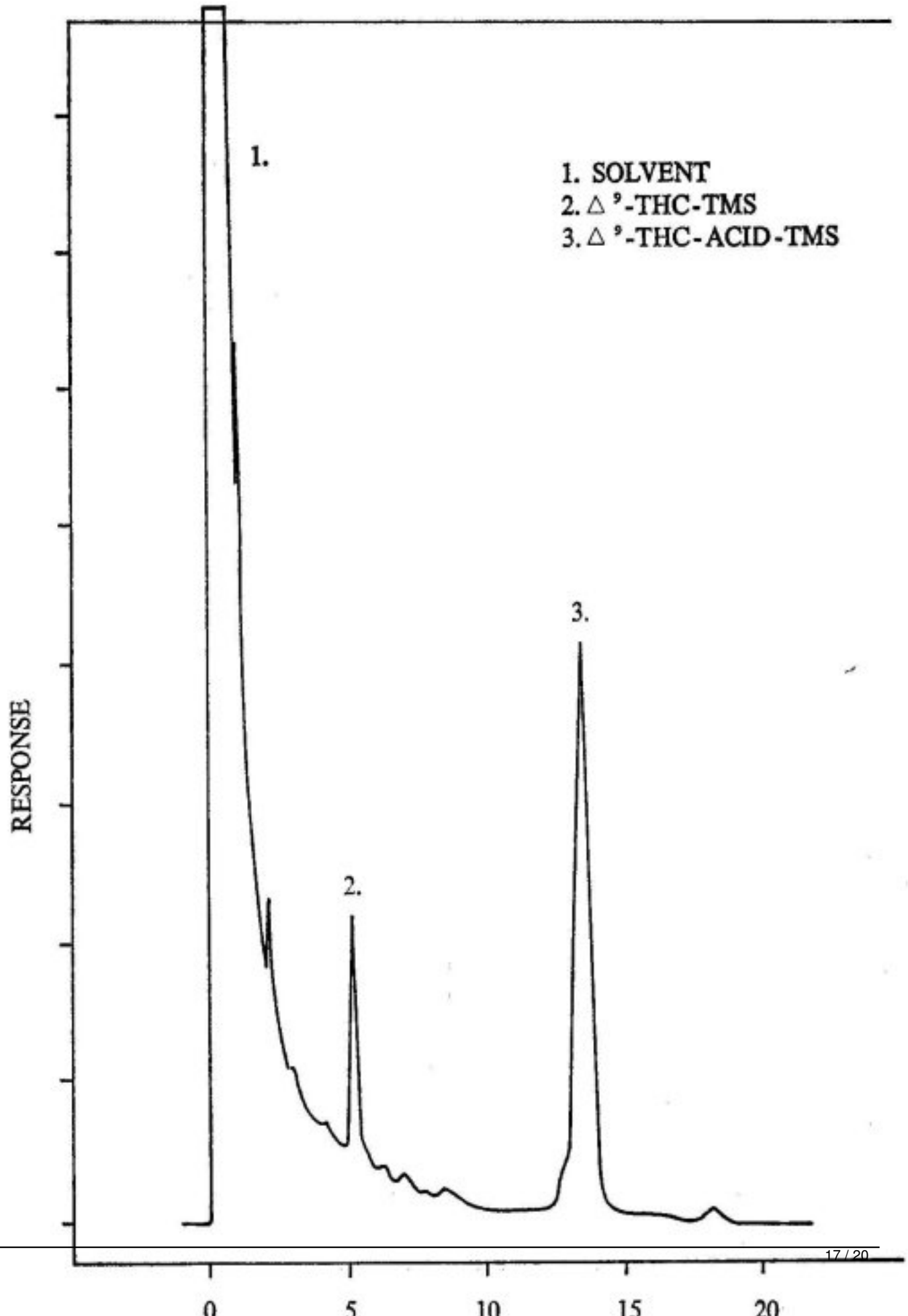
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1. SOLVENT
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3. Δ ⁹-THC-ACID-TMS
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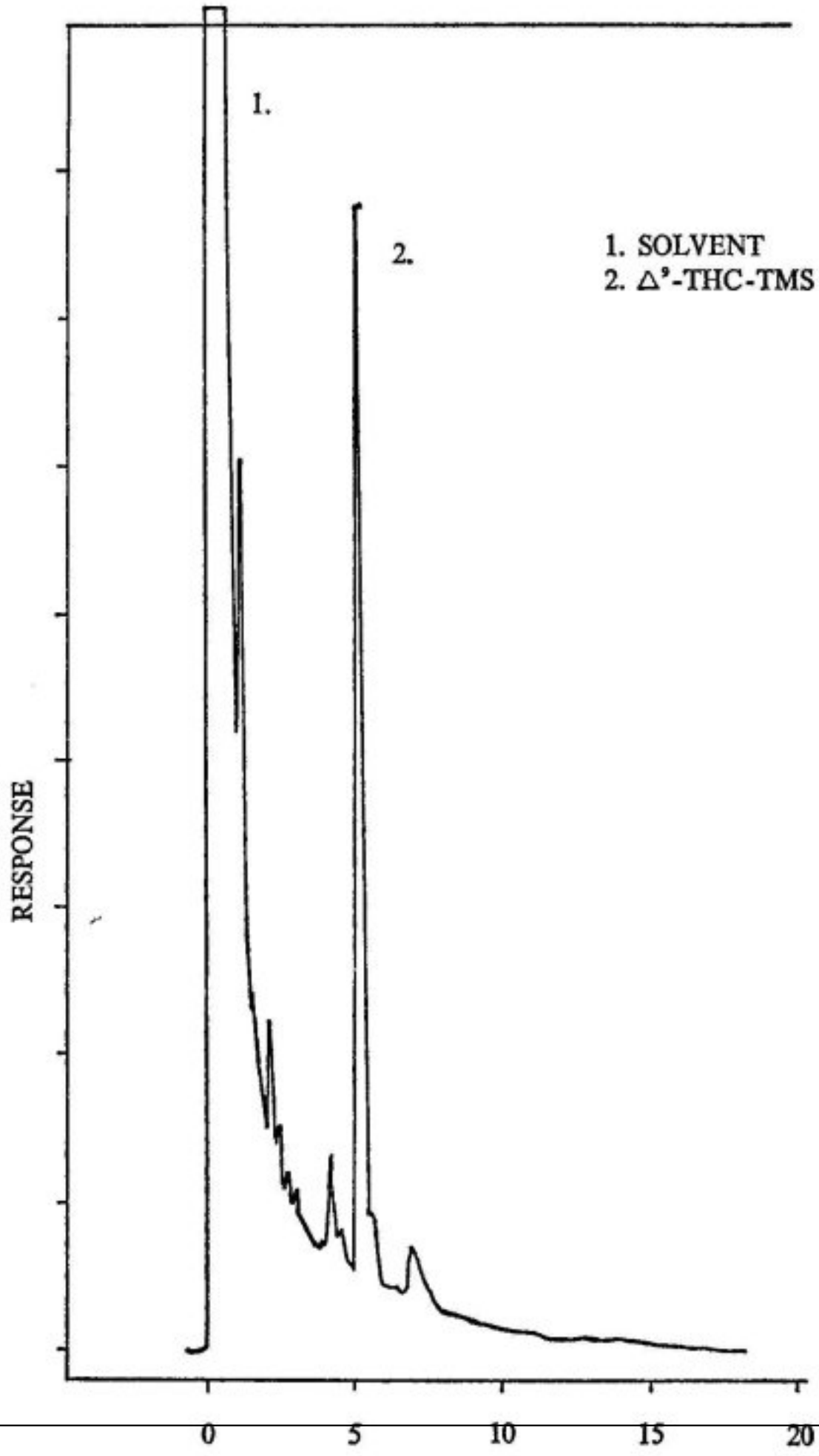
1.
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1. SOLVENT
2. Δ⁹-THC-TMS
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