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A Random Walk Through a Cannabis Field

R. MECHOULAM, W. A. DEVANE, A. BREUER AND J. ZAHALKA

Hebrew University Medical Faculty, Jerusalem 91 120, Israel

MECHOULAM, R., W. A. DEVANE, A. BREUER AND J. ZAHALKA. *A random walk through a cannabis field.* PHARMACOL BIOCHEM BEHAV **40**(3) 461–464, 1991.—The present overview covers various aspects of research going on in the Cannabis field in the Department of Natural Products at the Hebrew University. In the first part we discuss, and try to explain, the reason for the absence of the term Cannabis (and possibly also opium) in the Old Testament. In the second part we bring evidence that, contrary to widely held views, stereospecificity of cannabinoid action is extremely high, and in certain cases almost absolute. Previous results seem to have been due to impurities in the samples tested. (+)-Delta-1-THC, (+)-delta-6-THC and (+)-7-hyroxy-delta-6-THC, when purified sufficiently, exhibit activity of about 1% of that of the natural (−) enantiomers. A new labelled cannabinoid ligand has been prepared by catalytic reduction of (−)-7-hydroxy-delta-6-THC dimethylheptyl. The equatorial C-1 epimer obtained binds to the cannabinoid receptor with a K_I of 40 pM. This compound is one of the most active cannabinoids tested so far for binding to the canabinoid receptor, and may become an important tool in cannabinoid research.

Cannabis in the Bible Stereospecificity Ligand binding Catalytic hydrogenation Cannabinoids HU-210

THIS overview covers various aspects of our research in the field of Cannabis and presents some thoughts and ideas, which may, perhaps, help to advance this field.

Some Historical and Linguistic Notes

Cannabis has been used and misused over thousands of years. In the Middle East it was well known to the Assyrians, to the Egyptians, to the Scythians and, to a limited extent, to the Greeks (16). Yet there is no direct indication that it was known to or used by the ancient Jews. As a matter of fact, the Old Testament mentions very few drugs; even opium, a medicine of repute in the ancient world, is not in the Bible. Why? What is the reason, that a book which goes into great details of most aspects of life, sacred and profane, fails to mention drugs such as opium and Cannabis?

The Assyrians employed Cannabis apparently quite extensively in religious rites (as a drug to induce ecstasy) and as a medicinal drug (mostly in, what we would term today, neurological and psychiatric diseases) (2). The contacts between the Jews and Assyrians were prolonged and wide. During certain historical periods, the trade of Judea depended on Assyria; the cultural-religious impact of the Nineveh court and temples penetrated not only the royal and priestly class in Jerusalem but also the lower classes. Gradually, in the 7th century B.C., even the Assyrian cult of heavenly constellations was adopted and, in the Temple itself, an image of the Assyrian "queen of heaven" was erected. During the reigns of the Assyrian kings, Esarhaddon (681 to 669 B.C.) and Ashurbanipal (669 to 626 B.C.), the Jewish King Menasseh, who was their vassal, fought beside them in their wars. The influence of the kings of Nineveh ("the bloody city, full of lies and robbery") in Judea was immense. The political and cultural independence of Judea during that period was tenuous at best. It can be assumed that under these conditions, Assyrian drugs were known and used, at least among the ruling class, both as tools of religion and as medicines.

After the death of Ashurbanipal, the hedonistic kingdom of Assyria swiftly disappeared from history. the Jewish King Josiah (628 B.C.), during whose reign some of the books of the Old Testament were resurrected, and possibly rewritten, is known to have taken advantage of the Assyrian decay to remove vigorously all pagan influence from Jewish life and religious customs. Hashish, presumably a symbol of Assyrian moral laxity, would have been banned. This is, of course, an assumption, but it fits the historical background, and the political needs of an independent Judean state and its king. It explains the strange absence of the terms Cannabis and possibly opium in the sacred Jewish books.

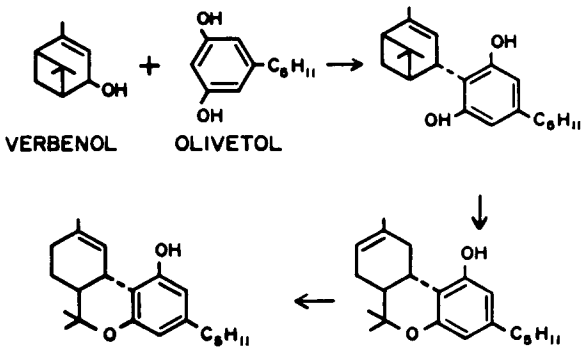
It is quite possible, however, that in one instance the ancient term for Cannabis is mentioned in the Bible. It is probable that "pannag", an unidentified product exported from, or transported through, Judea to Tyre, mentioned by the prophet Ezekiel (5,22), was in fact Cannabis. Presumably the Hebrew "pannag" was one of the original forms of the word (in Sanskrit "bhanga" and in Persian "bang"). In Hebrew and in other Oriental languages only the consonants form the basis of a word: the letters p and b are frequently interchangeable. The term "pannag" later apparently underwent a metathesis in Syriac ("qunnappa"), and classical Arabic ("kunnab"), finally becoming Cannabis in Greek.

The above examples show that modern research can still throw light on aspects of ancient history and social mores.

Now we would like to make a historical jump of circa 2500 years and present observations on some chemical, biochemical and pharmacological aspects of Cannabis research.

Novel Chemical and Pharmacological Aspects

Background. The 1960s brought a renaissance to Cannabis chemical research: the major active constituent, delta-1-tetrahydrocannabinol (delta-1-THC or delta-9-THC) was identified (6), most of the natural neutral and acidic cannabinoids were isolated and their structures were elucidated, the absolute configuration of this group of compounds was determined, and a reasonable scheme of biogenesis was put forward (15). Total syntheses were



Scheme I. Synthesis of (+)-delta-1-THC.

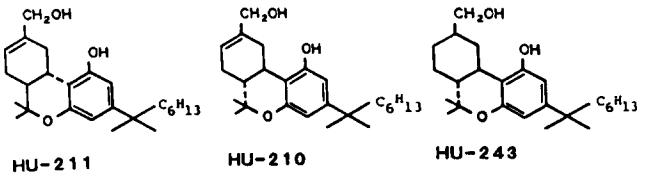
achieved for most cannabinoids (17,23). The basic structure-activity relationships as regards cannabimimetic activity were established (18,24). The first steps toward elucidating the biochemical mechanisms of Cannabis activity were taken. Pharmacological work in animals and man was initiated.

With the clarification of the chemical aspects of Cannabis, interest focused on cannabinoid pharmacology and behavioral effects, on the neurochemistry, neurophysiology, drug interactions and interactions with membrane components, alterations of macromolecular metabolism, alteration in prostaglandin synthesis and even cellular modifications as related to specific effects (13). Until recently, very little was known about the molecular mode of action of the cannabinoids. In 1988, a binding site for THC was identified (3,4). This binding site seems to be specific for cannabinoids, as a large number of natural ligands (such as acetylcholine, serotonin, norepinephrine, prostaglandins, etc.) as well as synthetic drugs do not bind to the receptor (1, 7, 14). The primary structure of the receptor has been determined following success in its cloning (14).

In the present lecture, we would like to present new data on the cannabinoids relating to a) stereospecificity of cannabinoid activity; and b) synthesis of a novel, easily available radiolabelled ligand for cannabinoid receptors.

Stereospecificity of cannabinoid activity. The natural delta-1-THC and delta-6-THC possess a (3R,4R) stereochemistry. The synthetic route which was developed by our laboratory for delta-1-THC and delta-6-THC nearly 20 years ago makes possible also the synthesis of the (3S,4S) enantiomers (Scheme I) (17). In tests for cannabimimetic activity done at several laboratories, (+)-delta-1-THC was ca. 13–20 times less active than the (−) natural enantiomer (11,24). These early results indicated pharmacologic enantiomeric preference rather than absolute stereoselectivity. The implications of these results in elucidating the biochemical basis of cannabinoids are considerable.

We have synthesized the enantiomers of the dimethylheptyl homologs of 7-hydroxy-delta-6-THC (20). In this synthesis, both an intermediate ketone and the final 7-OH-delta-6-THC-DMHs (HU-210) and (HU-211) (Scheme II) are crystalline compounds which can be obtained with absolute enantiomeric purity. In drug stimulus generalization tests, we found that, in the pigeon, (−)-7-OH-delta-6-THC-DMH (HU-210) was about 87 times more active than the natural (−) delta-1-THC. The (+)-7-OH-delta-6-THC-DMH enantiomer (HU-211) was inactive in the same animal at doses circa 4500 higher than those of the ED_{50} of the (−)-enantiomer, HU-210. The respective dose in the rat was circa 1000 times (10). Parallel results were obtained in the

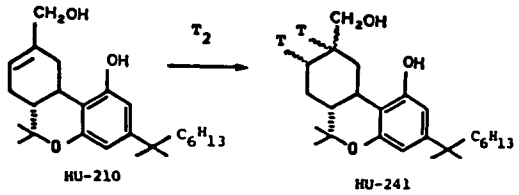


Scheme II. Various cannabinoids.

mouse ring test, in the rotarod test in rats, in the dog ataxia test and others (12,19). These results indicate in reality absolute stereospecificity and led us to reexamine the enantiomers of natural THC. In Scheme I, one of the intermediates, verbenol, is crystalline, and by repeated crystallizations it could be purified to 99% e.e., which leads to (+)-delta-6- and (+)-delta-1-THC of 99% e.e., as established by the formation, and gas chromatographic and NMR analysis of (S)-(+)-α-methoxy-α-(trifluoromethyl) phenyl-acetyl (MTPA) esters. (+)-7-Hydroxy-delta-6-THC prepared as described above for the dimethylheptyl homolog (HU-211) was also obtained in 99% e.e. as evidenced by HPLC analysis of its MTPA diester. Preliminary pharmacological tests indicate that indeed the (+)-THCs prepared in this manner exhibit activity of about 1% of that of the natural (−)-THCs. Further, more detailed, investigations are in progress. However the preliminary results tentatively indicate that any pharmacologic activity of the (+)-THC's is due to contamination by the (−)-THCs, i.e., stereospecificity in the THC series is absolute (or almost absolute).

On the basis of the absolute stereospecificity described above, we believe that cannabimimetic activity has strict stereochemical requirements, which indicates interaction with a chiral biochemical entity (enzyme, receptor site, etc.) and not just an unspecific action due to the high lipid solubility of the cannabinoids. It is possible, however, that as has been previously suggested (13) the pharmacological properties unique to the cannabinoid class of drugs are produced by the interaction of delta-1-THC with specific receptors, while cannabinoid-induced central nervous system (CNS) depression is a general phenomenon produced by nonspecific membrane interaction.

Synthesis of novel, easily available radiolabelled ligands for cannabinoid receptors. The recent identification of a cannabinoid receptor (3,4) made use of a radioligand whose structure is remote from that of the natural THC and which is not easily available. In order to make possible further work in this field, we have looked for novel, THC-type ligands which can be readily labelled. The availability of a second type of a radioactive ligand may also make possible the identification of subtypes of the cannabinoid receptor. We report now the synthesis of such a ligand.



Scheme III. Synthesis of a labelled cannabinoid ligand.

Several years ago we reported that reduction of the double bond of THC led to two C-1 epimers (18). The equatorial epimer was considerably more psychotropic than the axial one. The same observation was made with the metabolite 7-OH-THC: the two hexahydrocannabinols produced from it were not equipotent. The equatorial epimer was found to be not only much more active than the axial one, but also to be considerably more active in drug discrimination tests than delta-1-THC (circa 2–4 times in rats and 8–15 times in pigeons) (9). HU-210, mentioned above, competes fully for the specific binding of [^{3}H]CP-55940 (the cannabinoid tritiated ligand used until now for binding studies) to membranes from rat brain in heterologous displacement studies ($K_i = 234$ pM) (8). On the basis of the above observations, we assumed that hydrogenation (or tritiation) of HU-210 would produce compounds which bind to the cannabinoid receptor even better than HU-210. Indeed, hydrogenation of (−)-7-OH-delta-6-THC-DMC (HU-210) over platinum oxide led to a mixture of the C-1 epimers of (−)-7-OH-hexahydrocannabinols-DMH (HU-241, nontritiated) (Scheme III). In addition, some hydrogenolysed materials (i.e., compounds in which the hydroxyl group was removed) were obtained. These could be separated with ease by thin layer chromatography. When this reaction was done with tritium (at the Dimona research reactor laboratories) tritiated HU-241 was obtained with a specific activity of 12.6 Ci/mmol. Binding of [^{3}H]-HU-241 to rat cortical membranes was examined with the centrifugation assay previously described (3,4). Homologous displacement experiments yielded a K_d of 142 pM (n = 2) for HU-241 (Fig. 1). Heterologous displacement analyses yielded a K_i of 154 pM (n = 2) for HU-210. [^{3}H]-HU-241 should thus prove useful for characterizing the cannabinoid receptor and for testing new cannabinoid compounds. The corresponding (+) enantiomer of HU-210 (i.e., HU-211) was greater than 3000-fold less potent at binding to the receptor.

We are at present looking into hydrogenations with reagents which would lead to stereospecific reduction of the double bond, i.e., formation of the equatorial epimer only, in addition to absence of hydrogenolysis. The reagents described by Noyori's group (BINAP-Ru II complexes) (21) indeed cause reduction without any hydrogenolysis. However, the stereospecificity observed was low: the ratio of the equatorial C-1 epimer to the axial one was only 4:1. Preliminary reduction experiments with a mixture of Wilkinson's reagent (tris triphenylphosphine rhodium chloride) and Kagan's reagent (+,2,3-0 isopropylidene-2,3-dihydroxy-1,4-bis diphenylphosphinobutane) led to the desired epimer with essentially 100% stereospecificity. The compound obtained (HU-243, which is the equatorial C-1 epimer of the mixture depicted as HU-241, see above) binds to the receptor with a $K_i = 41$ pM (n = 3). This compound is one of the most active cannabinoids tested so far for binding to the cannabinoid receptor, and may become an important tool in cannabinoid research.

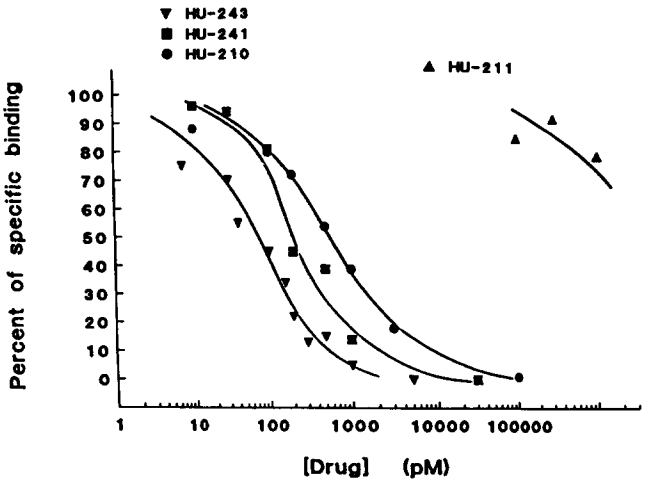


FIG. 1. Competitive inhibition of [^{3}H]-HU-241 binding by various synthetic cannabinoid drugs. Binding assays were performed as previously described (3,4). [^{3}H] HU-241 (70–100 pM) was incubated with P2 cortical membranes (20–50 μg protein) for 70 min at 30°C with either the indicated concentrations of drug or vehicle alone. Bound and free ligand were separated by centrifugation at 12,000 × g for 6 min. Data points represent the averages of triplicate determinations from single representative experiments. K_d and K_i values reported in the text were determined using the LIGAND program.

DISCUSSION

In the first part of this overview, we suggested that the virtual nonexistence of drug names in the Old Testament is probably due to political-religious considerations. If our thesis is correct, it suggests that the interplay of politics and drugs is not uniquely a modern issue.

In the second part of this overview, we addressed ourselves to several more modern and scientific topics: a) the stereospecificity of cannabinoid activity, and b) the synthesis of a new labelled ligand for the Cannabis receptor.

Relative to point a) (the stereospecificity of cannabinoid activity) we have presented new evidence emphasizing again that the stereospecificity of cannabinoids, as regards pharmacological activity, is apparently complete (or essentially complete), thus indicating that cannabinoid action is associated with binding, or interaction with biological substances (receptor sites, enzymes, etc.).

Relative to point b) (a new ligand for the cannabinoid receptor), we have described progress towards the preparation of novel labelled cannabinoid type ligands with very high binding affinity (down to $K_D = 40$ pm) to the cannabinoid receptor. Such ligands should be valuable tools in cannabinoid research.

Why do we have cannabinoid receptors (and also presumably endogenous cannabinoid ligands)? We can only speculate. Presumably, the Cannabis agonists act on *coordination of movement* via the cannabinoid receptors in the cerebellum and in the basal ganglia and on *memory* via the receptors in the hippocampus (1,7). In humans, these cannabinoid actions are rather marginal, and it seems that other activities should also be investigated.

Cannabis is used by man not for its actions on memory or movement coordination but for its actions on mood and emotions. Is it possible that the main task of the cannabinoid receptor (presumably via the putative natural cannabis ligand) is to modify our emotions, to serve as one of the links which transmit, or transform or translate objective or subjective events into perceptions and emotions? If correct, this action is probably interrelated with other receptors and transmitters; the serotonin system, the dopaminergic system, the endorphin system. But these systems have other major tasks as well, while the Cannabis system is not known (at present at least) to have major tasks in man. Anatomically this is possible. From published work we know that there are Cannabis receptors in the limbic system (1,7). There is general agreement that the limbic system occupies a central position in the neural mechanisms that govern behavior and emotions.

We know next to nothing on the chemistry of emotions. For thousands of years love and hate, happiness and sorrow have

been left to the poets.

What is happiness? It is hard to describe, which is why the Inferno of Dante is better known than his Paradiso.

Patricia Goedicke in her poem "Happiness" writes

"At first it fills you entirely,
Enveloped in it there's no room anywhere
For any other feeling,
It seems almost you could rise
Infinitely expandable, like hot air
Forever floating in a balloon." (25)

Can we translate this wonderful poem into biochemical cycles? Of course not. Let us hope, however, that through better understanding of Cannabis chemistry in the brain we may also approach the chemistry of emotions.

ACKNOWLEDGEMENT

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