Phytochemistry, Vol. 21, No. 1, pp. 67–69, 1982.
Printed in Great Britain.

0031–9422/82/010067–03 $03.00/0

THE BIOGENESIS OF CANNABINOIDS IN *CANNABIS SATIVA*

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(*Received* 4 *January* 1980)

Key Word Index—*Cannabis sativa*; Cannabinaceae; neutral cannabinoids; tetrahydrocannabinol; biogenesis.

Abstract—Cannabigerol and olivetol are incorporated into tetrahydrocannabinol and other neutral cannabinoids. Cannabidiol is not taken up in any of the neutral cannabinoids and thus it appears the latter are formed from neutral precursors.

INTRODUCTION

Since the first known work by Roxy [1], the biogenesis of cannabinoids has received little attention. The complexity of the matter and the lack of uniform results between different researchers explain the difficulty in drawing universal conclusions concerning the biogenesis of these compounds. Shoyama and co-workers [2] found that Mechoulam's hypothesis [3] dealing with cannabinoid biogenesis was right, in the main, but stated that only the cannabinoid acids were true natural products, the so-called neutral cannabinoids being but artefacts [2].

In preliminary work, we examined a *Cannabis sativa* strain lacking cannabidiol (CBD) and observed incorporation of both [^{14}C]olivetol and [^{14}C]olivetolic acid into cannabinoids. However, we did not comment on these results since we needed to complete our studies [4, 5]. This work deals with feeding experiments using [^{14}C]cannabigerol (CBG) and [^{14}C]cannabidiol. The results obtained allow us to discuss in much more detail the preceding observations [4] and relate them to the present work.

RESULTS AND DISCUSSION

Seeds of *Cannabis sativa* (South African UNC-255 strain) were used as a source and the resulting plants showed the presence of Δ^1-tetrahydrocannabinol (Δ^1-THC) and its *n*-propyl derivative (Δ^1-TCH-C_3) CBG, and cannabichromene as the major neutral cannabinoids. The acidic $^1\Delta$-THCA was the only major cannabinoid acid detected. Cannabidiol was absent as confirmed by TLC [6, 7], GC and MS using authentic samples. For this strain of *Cannabis sativa*, Turner and Paris have obtained similar results concerning the absence of cannabidiol [8, 9].

Feeding experiments were carried out when *Cannabis* plants were 21- to 31-days-old. All the labelled compounds were chemically synthesized. The synthesis of [side chain 2-^{14}C]olivetol has already been reported [4] and this product was used to obtain [^{14}C]CBG according to a previously reported synthesis [10]. [10-^{14}C]CBD was prepared according to Korte *et al.* [11].

The incorporation of olivetol was quite low as one would expect if neutral cannabinoids are artefacts and are

Table 1. Tracer experiments with various precursors

	Precursors							
	Olivetol (53.73×10^{-2})		Olivetolic acid (5.65×10^{-2})		Cannabigerol (0.54×10^{-2})		Cannabidiol (0.54)	
Cannabinoids extracted	Sp. act. DPM/mM	%	Sp. act.	%	Sp. act.	%	Sp. act.	%
THC	7.23	0.052	4.30	0.14	74.98	0.78	0	0
THCA	0	0	0	0	0	0	0	0
CBG	5.89	0.014	2.29	0.039	163.6	0.25	0	0
CBN	5.29	0.06	0.26	0.006	33.37	0.44	0	0
CBCh	5.62	0.015	7.30	0.26	55.60	0.22	0	0

THC, Tetrahydrocannabinol; THCA, tetrahydrocannabinolic acid; CBG, cannabigerol; CBN, cannabinol; CBCh, cannabichromene; CBD, cannabidiol.

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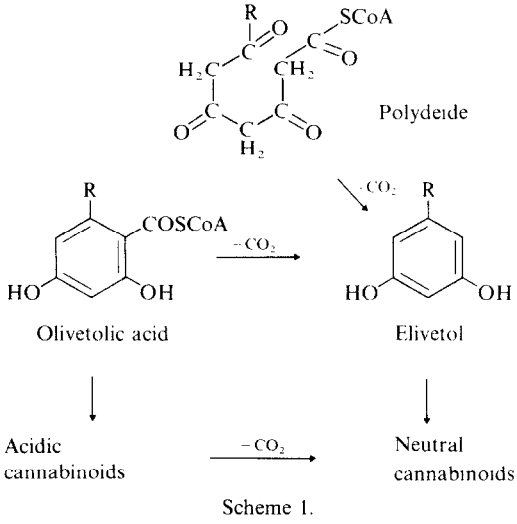
formed during harvesting and storage. It seems to us that olivetol should be one of the primary precursors of neutral cannabinoids. The most striking feature of our work is that Δ^1-THC-C_3 was never labelled, whatever precursor is used. We thus assume that *n*-propylcannabinoids are not formed by a shortening of the amyl side chain of the more common cannabinoids.

When olivetolic acid was used both Δ^1-THC and Δ^1-THCA were labelled; the former being less labelled than the acid. This should mean that an acidic precursor mainly yields acidic compounds. These may then be further decarboxylated to yield the neutral compounds. We believe that decarboxylation of acidic cannabinoids is a continuing process yielding neutral cannabinoids and may occur at an early stage and continue throughout the plant's life (Scheme 1).



Scheme 1.

Incorporation of CBG was higher than that of either olivetol or olivetolic acid as might be expected because it is close to other cannabinoids. This fact undoubtedly explains its higher incorporation into all neutral cannabinoids we have examined.

Surprisingly CBD is not taken up at all. Why such a natural product is not incorporated whereas olivetol and olivetolic acid are is difficult to explain. Neither of the latter polyketide precursors have been isolated so far from any known strain of *Cannabis*.

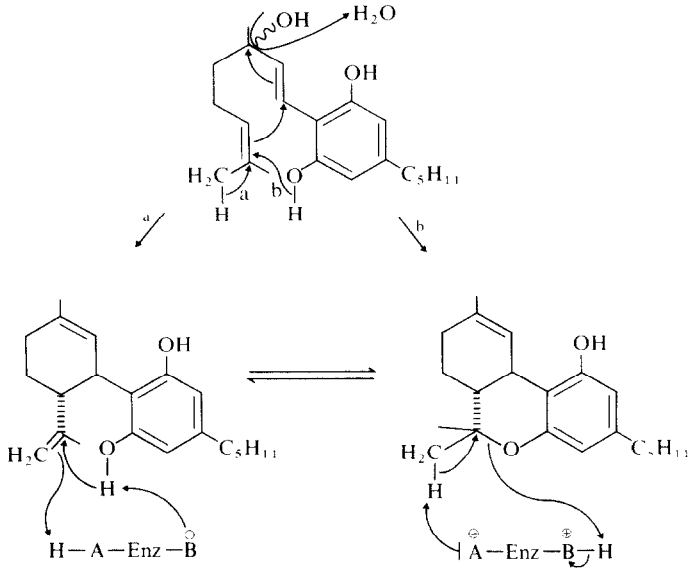
It seems possible that an enzymatic block which prevents the formation of CBD in this strain of *Cannabis* may be responsible for hindering its incorporation. We believe that CBD and THC may be formed through separate but reversible pathways (Scheme 2). During this process an amphoteric enzyme should allow interconversion of CBD and vice versa. The pathway leading from THC to CBD is absent in the strain of *Cannabis sativa* we used. According to these results we believe that neutral cannabinoids are formed through two separate processes. The first, involving the decarboxylation of cannabinoid acids, is probably important during storage; the second starts from neutral precursors as our results show and could occur throughout the lifetime of the plant.

EXPERIMENTAL

Incorporation of precursors and extraction. Precursors were dissolved in 10 ml Me_2CO and diluted to 200 ml with H_2O and fed to the plants. Ten plants were used for each experiment and kept for 6 days in the radioactive soln. Radioactivity was determined by liquid scintillation counting with toluene as an internal standard. The roots and parts of the cuttings which were dipped into the radioactive soln were cut off and discarded so as not to interfere with counting. The remaining material was chilled in liquid nitrogen and extracted in cold petrol (bp 60–80°).

Isolation of cannabinoids. Prep. TLC (Si gel F_{254}) was used with toluene–Et_2O (4:1) as the developing system along with authentic samples of THC, CBG and CBCh [12].

Identification of extracted cannabinoids. Three TLC systems were used [6, 7] along with the process described below. Si gel



Scheme 2.

F_{254} was dipped into diethylamine and dried in an oven at 100° for 15 min; development was performed with toluene–Et_2O (4:1) and spots were visualized by means of a spray (0.1% soln of Fast Salt B) using authentic samples, the characteristics of which were checked by IR, GC, NMR and MS analysis [13].

Acknowledgements—We are grateful to V. Vande Velde and Prof. Mechoulam for supplying CBG and CBD. The gift of Haschisch tablets from the Police Belge des Stupéfiants is appreciated. We also are indebted to Prof. M. Paris and Miss F. Boucher for seeds of UNC-255.

REFERENCES

1. Roxy, Ru Jien Ni (1963) Dissertation, University of Minnesota.
2. Shoyama, Y., Yagi, M., Nishioka, I. and Yamauchi, T. (1975) *Phytochemistry* **14**, 2189.
3. Mechoulam, R. (1970) *Science* **168**, 1159.
4. Schmit, J. P., Vande Velde, V. and Piraux, M. (1975) *J. Labelled Compds.* **II**, 51.
5. Kajima, M., Vande Velde, V. and Piraux, M. (1978) *Bioorg. Chem.* **I**, 163.
6. Gaoni, Y. and Mechoulam, R. (1971) *J. Am. Chem. Soc.* **93**, 217.
7. Aramaki, H., Tomiyasu, N. and Yoshimura, H. (1968) *Chem. Pharm. Bull.* **16**, 822.
8. Turner, C. E. and Hadley, K. (1973) *J. Pharm. Sci.* **62**, 251.
9. Paris, M., Boucher, F. and Cosson, L. (1975) *Plant. Méd. et Phytothér.*, **IX**, 136.
10. Mechoulam, R. and Yagen, B. (1969) *Tetrahedron Letters* 5349.
11. Korte, F., Dlugosch, E. and Claussen, U. (1966) *Justus Liebigs Ann. Chem.* **693**, 165.
12. Kane, V. V. and Razdan, R. K. (1968) *J. Am. Chem. Soc.* **90**, 6551.
13. Mechoulam, R. and Gaoni, Y. (1967) *Fortschr. Chem. Org. Naturst.* **25**, 175.