the active period in *Rana esculenta* corresponds with the beginning of the fat body renewal. These results are in contrast to the storage of depot substances in the liver, which begins later[9]. However, the triglyceride reaches its maximum in October in the fat body as well as in the liver. The continuous decrease in the fat body's triglyceride content from October to May clearly shows the decisive role of this depot organ for the energy supply during the winter fasting period of *Rana esculenta*. The distinct decrease in the fat body triglyceride ends in March. At that time, this substrate reaches its second maximum in the liver[9]. That means that at least at this time triglycerides derived from the fat body are accumulated in the liver. It is possible that they are converted or incorporated in greater complexes, taking into account the spawning season.

The protein content of the fat body undergoes a comparable annual rhythm to that of the triglycerides (figure C). The mass of the incorporated protein seems to be negligible, serving as an additional energy supply. It is more likely that the protein concentration is in direct correlation with the mechanism of triglyceride incorporation and release.

Surprisingly the fat body contains a relativly high amount of glycogen (figure C), which is too high to consider the fat body in *Rana esculenta* only as a triglyceride storage organ. This correlation strikingly resembles the brown adipose tissue in higher vertebrates. Because the glycogen maximum is found in August, in contrast to the triglyceride's in October, there could be different physiological demands as far as the energy supply is concerned. The glycogen increase in the fat body, however, seems to be less relevant in the period from February until May, because at this time the fat body weight strongly decreases. Although fat body weight increases in any case strongly from May by triglyceride incorporation, there is an additional glycogen level increase. In this correlation, it is specially striking that, during the fat body glycogen increase, the liver synthesizes no glycogen depot[9]. The liver glycogen does not increase before fat body glycogen decreases in September. The physiological background of this phenomenon can only be a subject of speculation at this time.

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Microbiological oxidation of the pentyl side chain of cannabinoids

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Summary. Syncephalastrum racemosum ATCC 18192 and *Mycobacterium rhodochrous* ATCC 19067 partially degrade the n-pentyl side chain of cannabidiol, cannabinol, Δ^8-tetrahydrocannabinol and Δ^9-tetrahydrocannabinol. Carboxylic acid and alcohol side chain derivatives are major metabolites.

We have screened more than 100 species of fungi and bacteria for the ability to transform 4 common cannabinoids, namely cannabidiol (CBD), cannabinol (CBN), Δ^8-tetrahydrocannabinol (Δ^8-THC) and Δ^9-tetrahydrocannabinol (Δ^9-THC). The 4 cannabinoids studied are known to possess a variety of potentially useful pharmacological activities in addition to the psychotropic effects for which *Cannabis sativa* L. and its preparations receive popular use[2]. Such physiological actions as anticonvulsant, antidepressant, hypotensive, bronchodilation and lowering of intraocular pressure have led a number of groups to investigate the possible development of useful medicinal agents from the growing ranks of naturally occurring and synthetic cannabinoids. A major emphasis in the study of the effects of these characteristic marihuana components on humans has been the elucidation of the routes of metabolism. In mammalian systems transformation reportedly proceeds by initial monohydroxylation at allylic positions in the monoterpene portion of the cannabinoid molecule or on the carbons of the aliphatic side chain. The subsequent introduction of additional hydroxyl groups and the further oxidation of some intermediate alcohols to aldehydes, ketones or carboxylic acids lead to a complex mixture of polar metabolites[3], which may be further complicated by conjugation. Because certain of these polar metabolites have not been produced in good yields by standard organic synthetic methods, we decided to investigate microbial transformation as a means of producing certain metabolites in quantities sufficient for animal testing and other studies[4]. In addition, some of the microbially catalyzed reactions may be useful tools in the preparation of novel synthetic cannabinoids.

Materials and methods. All screening and small scale production studies were carried out in shaken Erlenmeyer flasks (25 ml culture broth per 125-ml flask, or 100 ml per 500-ml flask) incubated at 25 °C and 250 rpm. The medium routinely used for the cultivation of the 2 organisms discussed in this study is a yeast-malt extract broth (Difco). Inoculation of sterilized medium is achieved by aseptic transfer of an aqueous suspension of surface growth (fungal conidia or bacterial cells) from agar slants into the culture flasks. After abundant growth is obtained (usually 24–48 h), the cannabinoid (obtained from the National Institute on Drug Abuse) was added as a small volume aliquot of a concentrated ethanolic solution, at a level of 10 or 20 mg/100 ml culture. Following suitable incubation periods, varying from 1 to 6 days, the cultures were adjusted to pH 3 and rapidly extracted with 3 equal portions of chloroform or ethyl acetate. Extracts containing cannabinoids and metabolites were dried over anhydrous sodium sulfate, concentrated under reduced pressure and examined by TLC[4].

Fig. 1. Transformation products formed by incubation of cannabidiol (**I**) with vegetative cultures of *Syncephalastrum racemosum* ATCC 18192.

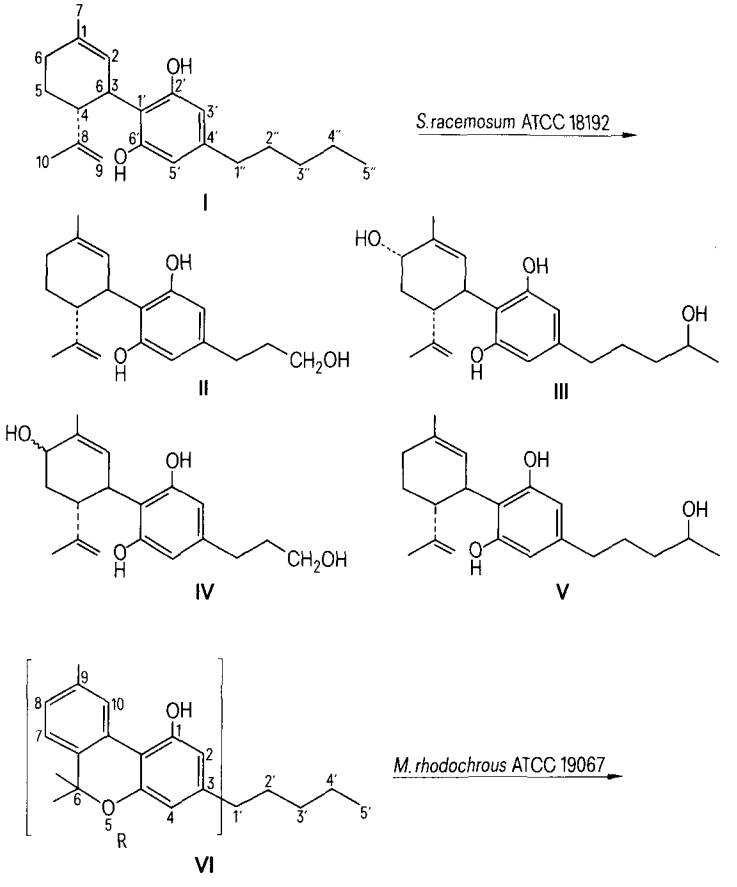
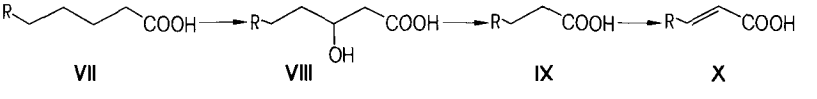


Fig. 2. Transformation products formed by incubation of cannabinol (**VI**) with cultures of *Mycobacterium rhodochrous* ATCC 19067.



Microorganisms which appeared to be producing cannabinoid metabolites were cultivated on a larger scale, and extracts of incubations were subjected to preliminary column chromatographic and preparative TLC techniques. Partially purified fractions were then analyzed by gas chromatography (gc) and gas chromatography-mass spectrometry (gc-ms). Whenever possible, electron impact (ei) and chemical ionization (ci) low resolution mass spectra were obtained on both underivatized metabolites and their trimethylsilyl (TMS) ethers. Subsequent scale up and isolation were next attempted in order to obtain quantities of metabolites sufficient for ^{1}H-nuclear magnetic resonance (pmr), infrared (ir) and other physical and chemical studies.

Results and discussion. Syncephalastrum racemosum ATCC 18192, a fungal organism which has been used industrially in steroid hydroxylations, was found to produce a variety of metabolites when incubated with each of the 4 cannabinoids. The highest yields of transformation products are formed when CBD (**I**) is incubated with growing cultures of *S. racemosum* for a 3-day period. Metabolites **II** through **V** are shown in figure 1 in order of decreasing quantitiy produced. The most abundant product has a 3-carbon side chain which terminates in a primary alcohol, namely 4″,5″-bisnor-3″-hydroxy-CBD (**II**). The structure assignment is supported by both ei and ci low resolution ms, high resolution ms, 90 MHz pmr, and ir. This unusual compound, which is unlike any of the metabolites reported thus far from mammalian systems, can be isolated in approximately 5% yield, based on the amount of CBD initially added to the cultures. Metabolite **III**, 4″,6α-dihydroxy-CBD, exhibits a characteristic mass spectral fragmentation, both TMS-derivatized and underivatized, identical with that of a dihydroxy CBD metabolite reported to be formed by rat liver[5]. The structural assignment was further confirmed by analysis of the 90 MHz pmr-spectrum.

The 4″,5″-bisnor-3″,6-dihydroxy-CBD (**IV**) was isolated in small mg amounts. Subterminal (ω-1) hydroxylation of the pentyl side chain results in the formation of 4″-hydroxy-CBD (**V**). We have previously reported the 4″-hydroxy metabolites as the major transformation products of CBD, CBN, Δ^8-THC and Δ^9-THC by cultures of *S. racemosum* grown in Mycophil (BBL) broth[4]. The 4″-hydroxy compound has been reported as the major side chain hydroxylated metabolite of CBD formed by rat liver[6]. In addition, side chain hydroxylated cannabinoid metabolites have been reported in a number of other animal systems[7].

Mycobacterium rhodochrous ATCC 19067 is a bacterium which is known to oxidize hydrocarbons. Incubation of any of the 4 cannabinoids with growing cultures or washed cell suspensions results in the formation of a variety of acidic metabolites. Figure 2 shows the microbial biotransformation products formed by incubating CBN (**VI**) with cultures of *M. rhodochrous* for 3 days. CBN was chosen for illustrative purposes, since the sequence of degradation appears to

be most complete for that cannabinoid. The acids in figure 2 were obtained in a single gc-ms analysis of oxidation products of CBN. A partially purified culture extract was silylated prior to the gc-ms analysis. CBN-5′-oic acid (**VII**), 3′-hydroxy-CBN-5′-oic acid (**VIII**), CBN-3′-oic acid (**IX**), and the 1′,2′-unsaturated CBN-3′-oic acid (**X**) are the identifiable products, with the CBN-3′-oic acid (**IX**) being the major metabolite. *M. rhodochrous,* therefore, appears to degrade the side chain of CBN by terminal oxidation to a 5′-carboxylic acid, followed by 1 or 2 cycles of the beta-oxidation process. Incubation of CBD with *M. rhodochrous* leads to the formation of CBD-5″-oic acid and CBD-3″-oic acid. Depending on the length of the incubation period and other variables, either the 3″- or the 5″-oic acid may accumulate in greater abundance. The ms fragmentation patterns of the CBD acid metabolites are well defined and very diagnostic for the identification of the acids. The major product formed from the incubation of Δ^8-THC with *M. rhodochrous* is the Δ^8-THC-3′-oic acid, and a small amount of the 1′,2′-unsaturated Δ^8-THC-3′-oic acid is also detectable. Numerous side chain carboxylic acid metabolites of cannabinoids have also been identified in mammalian systems, in which the odd-numbered 1′-,3′- and 5′-oic acids are the most common[8–11]. In addition, other workers have reported the production of carboxylic acid side chain degradation products of the synthetic cannabinoid nabilone by the bacterium *Nocardia salmonicolor*[12].

In conclusion, we have identified 2 different microorganisms which are capable of partially degrading the cannabinoid pentyl side chain by removing the 2 terminal carbons. *M. rhodochrous* gives the same type of products as are obtained in the animal systems which are known to oxidize the side chain to form carboxylic acids. *S. racemosum* acts upon the pentyl side chain by a subterminal oxidation process. It is likely that both organisms metabolize the cannabinoid side chain as if it were the terminus of a long straight chain hydrocarbon.

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Dominance and hierarchy in *Polistes gallicus* colonies attained through photoelectric properties

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Summary. There is a clear difference between the photoconductive properties of the workers and the females in *Polistes gallicus* wasp colonies. It is suggested that the dominance order in these colonies is based also on their photoconductive properties, which may have organizational and orientational values.

A dominance system has been shown to exist among females of the paper wasp *Polistes gallicus,* so that when 2 or more females found a nest in the spring, one comes to function as the egg layer, while the others assume a subordinate role[2,3]. Pardi[4,5] discovered that the dominant, fecund female establishes her position and controls the other females by direct aggressive behaviour.

Suspecting that the dominance and hierarchy among the females of a *Polistes* colony might have some organic basis, we decided to study the photoelectric properties of the cuticle of various wasps. In previous papers[6,7], we reported that the yellow strips on the gaster and frons of hornet workers *(Vespa orientalis)* are photoconductive. This photoconductive phenomenon occurred following preliminary irradiation of the workers with the light. The present paper describes attempts to measure the photoelectric properties of various members of several *Polistes gallicus* colonies in order to ascertain whether possible differences in the properties of their cuticle might determine the hierarchy of females (i.e. the dominance of the so-called α-females over the subordinate β-, γ-, ... ω-females), and the differences between the α-female and the workers in the colony.

2 series of experiments were performed to determine 2 points: a) the change in the cuticular resistance under short (2 min) illumination followed by 20 min darkness; b) the change in resistance under prolonged illumination (10 min) followed by a period of alternating light-darkness (30–30 sec of each). The test insects were females (α, β, and γ) and workers from 7 polygynous colonies of *P. gallicus* collected from Mosciano (Florence), Italy. The order dominance in the colonies was observed during the active season in the Department of Zoology of Florence University. Our measurements pertained to the abdominal segments only. The resistance was measured with a Keithley Digital Electrometer Model 616, having a sensitivity in the range of 10^5 to 10^{12} Ohm. Hookup of the cuticle strips to the electrometer was accomplished with a tungsten wire, 0.05–0.1 mm in diameter, both ends of which were smeared with a small amount of colloidal silver paint. The source of light was a microscope incandescent white light bulb, 100 W and 24 V.