Identification of (3R,4R)-$\Delta^{1(6)}$-tetrahydrocannabinol as an isolation artefact of cannabinoid acids formed by callus cultures of *Cannabis sativa* L.

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Summary. For the first time we have isolated a major psychoactive cannabinoid, (3R,4R)-$\Delta^{1(6)}$-tetrahydrocannabinol **3** from callus cultures of *Cannabis sativa* L. **3** was obtained as an artefact of the actually formed (3R,4R)-Δ^{1}-tetrahydrocannabinol-3′- and/or 5′-carboxylic acids **1** and **2** by subjecting the culture material to a decarboxylation step prior to extraction. No attempt was made to isolate acids **1** and **2**. The identity of **3** was confirmed by comparison with an authentic sample of $\Delta^{1(6)}$-tetrahydrocannabinol. Culture conditions, isolation procedure and identification of the cannabinoid are described.

The formation of cannabinoids in tissue cultures of *Cannabis sativa* L. is of special interest for investigating the biogenesis of cannabinoid structures since incorporation experiments can be much better performed in tissue cultures than in the intact plant. The elucidation of the biogenesis of cannabinoids has proceeded little beyond the hypothetical biogenetic scheme proposed by Mechoulam[3] and only 2 investigations have been reported[4,5]. The attempts so far reported to induce the formation of cannabinoids in callus cultures of *C. sativa*[6,7] have been unsuccessful. This failure may have been due in part to the choice of and/or concentration of growth regulators used to induce the formation of cannabinoids, and in part to the extraction procedures employed to isolate potentially cannabinoid compounds; these did not take into account the fact that under culture conditions the biogenetic precursors Δ^{1}-tetrahydrocannabinol-3′- and/or 5′-carboxylic acids (**1** and **2**) are formed rather than the neutral cannabinoids themselves. In the plant, acids **1** and **2** are converted to the neutral cannabinoids by light and heat.

As expected, extraction of culture material dried in vacuo at 23 °C with cold ethanol did not yield any neutral cannabinoids. Therefore we developed a different extraction procedure including a decarboxylation step to convert acids **1** and **2** to $\Delta^{1(6)}$-tetrahydrocannabinol (=$\Delta^{1(6)}$-THC) **3** with simultaneous isomerization of the double bond from 1(2) to 1(6) position (fig. 1).

$\Delta^{1(6)}$-THC-3′-carboxylic acid **4** was synthesized as described[8], applied to cellulose and heated for 15 min under Ar at 120 °C. Extraction with hot ethanol yielded quantitatively $\Delta^{1(6)}$-THC **3** the identity of which was confirmed by ^{1}H-NMR and mass spectroscopy. The callus cultures were derived from the epicotyls of seedlings from a *C. sativa* strain *(C. sativa* var. *indica)* and cultivated on several variations of B 5 medium (according to Gamborg et al.[9]) as listed in the table. The cultures were harvested, dried, subjected to decarboxylating conditions, extracted (details below) and subjected to TLC. Phenolic or cannabinoid material was detected by spraying with a Fast Blue B salt (FBS) solution. FBS positive material was observed in cultures grown on media B5-1, B5-2 and B5-3, media B5-1 and B5-3 giving the best results.

For the identification of the FBS-positive compounds, cultures grown on B5-1 medium at 23 °C under permanent

Variations of B5 medium

Medium	Growth regulators (mg/l)			
	2,4-D	IAA	NAA	K
B5-1	2.0	0.5	0.5	0.2
B5-2	–	–	5.0	–
B5-3	1.0	–	–	–
B5-4	1.0	–	–	0.1
B5-5	1.0	–	–	0.5
B5-6	1.0	–	–	5.0
B5-7	–	–	1.0	–
B5-8	–	–	2.0	–
B5-9	–	–	10.0	–

2,4-D, 2,4-dichlorophenoxyacetic acid; IAA, indoleacetic acid; NAA, naphthyl-1-acetic acid; K, kinetine. Composition of B5 medium see Gamborg et al.[9].

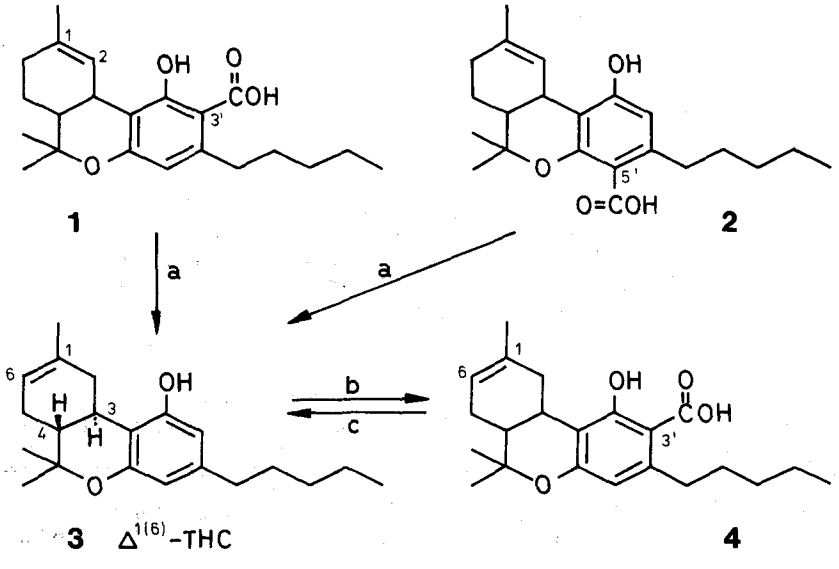

Figure 1. Structures of Δ^{1}-THC-3′-carboxylic acid **1**, Δ^{1}-THC-5′-carboxylic acid **2**, $\Delta^{1(6)}$-THC **3** and $\Delta^{1(6)}$-THC-3′-carboxylic acid **4**. a) **1** and **2** are transformed to **3** by decarboxylation and extraction procedure. b) Synthesis of **4** from **3** ($CH_3OMgCOOCH_3$/DMF)[8]. c) Reconversion of acid **4** to **3** (Ar, 120 °C, 15 min).

illumination were harvested after 30 days, dried at 65 °C for 24 h (about 2.3 g dry wt), subjected to the decarboxylation procedure and extracted continuously with 100 ml absolute ethanol under reflux for 1 h. The solvent was removed in vacuo, and the residue taken up in a small amount of ethanol and analyzed by TLC. Comparison with authentic samples of cannabinoids, $\Delta^{1(6)}$-THC **3**, Δ^{1}-THC, cannabidiol, cannabinol, revealed the presence of a FBS-positive compound with the same R_f-value as $\Delta^{1(6)}$-THC **3** in 3 solvent systems (petroleum ether/ether 4:1; petroleum ether/CH_2Cl_2 1:1; $CHCl_3$). The cannabinoid was purified by chromatography on Sephadex LH-20 (column 1.5×90 cm; eluent: petroleum ether/$CHCl_3$/ethanol 10:10:1) as described earlier[10]. The major compound obtained in a yield of 150 µg (6.5×10^{-3}% of dry wt) was shown to be identical to $\Delta^{1(6)}$-THC **3** by mass spectroscopy and gas liquid chromatography on SE-30 and OV-17.

Growth kinetics of the callus cultures were studied on B5-3 medium (fig. 2). The formation of acids **1** and/or **2**, determined as $\Delta^{1(6)}$-THC **3** correlated closely with the exponential growth phase of the cultures and the concentration of **3** declined sharply as growth stagnation.

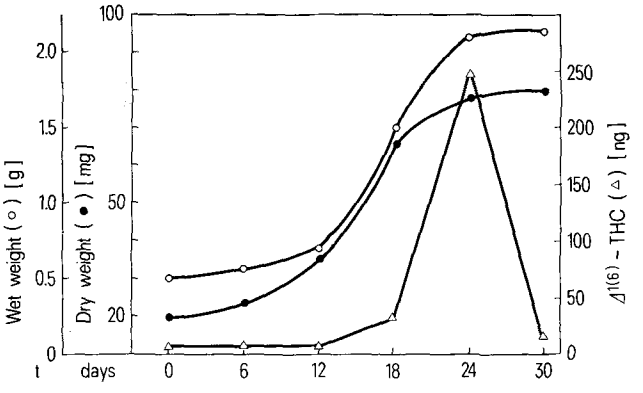

Figure 2. Correlation of growth period (days), wet weight (○) and dry weight (●) of callus cultures of *C. sativa* with the concentration of $\Delta^{1(6)}$-THC (△) **3**, taken as a measure for acids **1** and/or **2**. All values are averaged from 2 determinations.

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Hypolipidemic action of onion and garlic unsaturated oils in sucrose fed rats over a two-month period

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Summary. The feeding of a high-sucrose diet to normal rats for a period of 2 months increased serum and tissue cholesterol and triglycerides very significantly. Simultaneous feeding of the unsaturated oils of onion or garlic with the sucrose diet counterated these effects of sucrose. However, along with the lipid-reducing effects, a small but significant tissue-protein reducing effect of the oils were also observed. The hypolipidemic action of the oils may be related to their action on both lipid and protein synthesis.

Certain medicinal effects of onion and garlic and their volatile unsaturated principles have been observed before[2-4]. In 2 separate studies it was shown that aqueous extracts of these vegetables could counteract the hyperlipidemic effects of sucrose in normal rabbits[5,6]. Sucrose, which is a food with a high calorie-yield, has been observed to be responsible for increasing certain enzymes, such as glycerokinase, alpha-glycerophosphate dehydrogenase[7] and lipogenic enzymes[8]. Plasma glycerides are increased to varying degrees by sucrose depending on age, obesity and sex[9,10]. Over-consumption of sucrose leads to increased conversion of both glucose and fructose to triglyceride fatty acids[11,12]. Hyperlipidemia is a high risk factor in heart diseases[13,14]. It is found that the incidence of heart diseases is low in countries where onion and garlic are widely used; the hypolipidemic actions of onion and garlic are pertinent in this context. Temple[15] observed that the hypocholesterolemic action of garlic is due to its sulfur compounds. Gas-chromatographic analysis of steam-volatile fractions (oils) of onion and garlic[16] showed that they are composed of allyl propyldisulphide (C_3H_5–S–S–C_3H_7) and diallyl disulphide (C_3H_5–S–S–C_3H_5) respectively. The present study was designed to investigate the hypolipidemic actions of these two unsaturated oils in sucrose-fed rats.

Materials and methods. Allyl propyl disulphide and diallyl disulphide were prepared according to a published modification[17] of the method of Platenius[18] from fresh onion and garlic respectively. Male Wistar rats (average weight 150 g) were used for the experiments. They were divided into 4 groups of 6 each. 1 group was maintained ad libitum on a rat diet supplied by Pfizer (Kaduna, Nigeria). The composition of the diet was carbohydrate 73%, protein 16%, fat 3%, fiber 5%, minerals 2% and vitamin supplements 1%. This group was kept as a control group. The other 3 groups i.e. groups 2, 3 and 4 were given a sucrose-rich diet[19] ad libitum. This diet was composed of 73% sucrose, 20% milk powder, 5% fiber (Millet husk) and 2% salt mixture enriched with vitamins. The 2nd group of rats were kept as a sucrose control group. The 3rd group of rats were given the unsaturated onion oil (100 mg/kg/day) and the 4th group was given the unsaturated garlic oil, in the same dose as above, as a saline suspension through stomach tubes. Feeding of the sucrose rich diet and the 2 unsaturated oils was continued for a period of 2 months. At the end of the 60 th day the rats were sacrificed and their blood, livers, and kidneys were collected for various estimations. Blood sugar was estimated by the method of Asatoor and King[20]. Serum albumin and total protein were determined by the method