# Isolation of other substances from the leaves of Indian cannabis sativa L.

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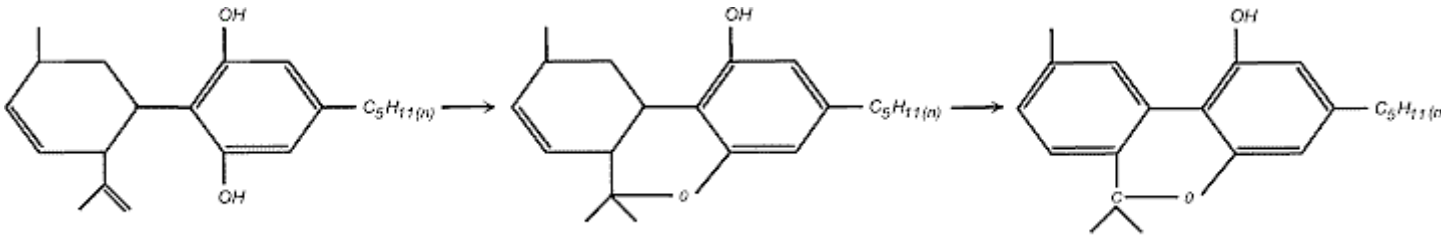
*PRELIMINARY NOTICE*

In the past two decades, attention has also been paid to the isolation and toxicology of the substances contained in the Cannabis sativa plant and especially its indica variant. The following substances were isolated: cannabinol, cannabidiol, tetrahydrocannabinol, quebrachitol (1 - inositol monomethyl ether), p-cymol, humulene (alpha-caryophyllene), so-called cannabol of phenotic character, which provides a well-crystallizing ester with azobenzenecarbonic acid chloride and an unidentified, optically active material of an etheric nature. In addition, a number of less important substances were isolated from the individual parts of the plant, collected in Wehmer's compendium. The change in the carotene content present in Cannabis sativa has been studied by Lebedev.

A very high acidic fraction (10 - 20 %) in the isolation of cannabinol from hashish, an intoxicating substance isolated from Indian cannabis, is mentioned in two studies, but none of them deals with the isolation of the substances contained in this fraction. Bergel's work only states that these are higher fatty acids.

Of the substances isolated and identified so far, cannabinol (III), cannabidiol (I) and tetrahydrocannabinol (II) have proven to be pharmacologically interesting; The substances have even been prepared artificially and have the following formulas (17 A-E, 18A-C, 19A-C):



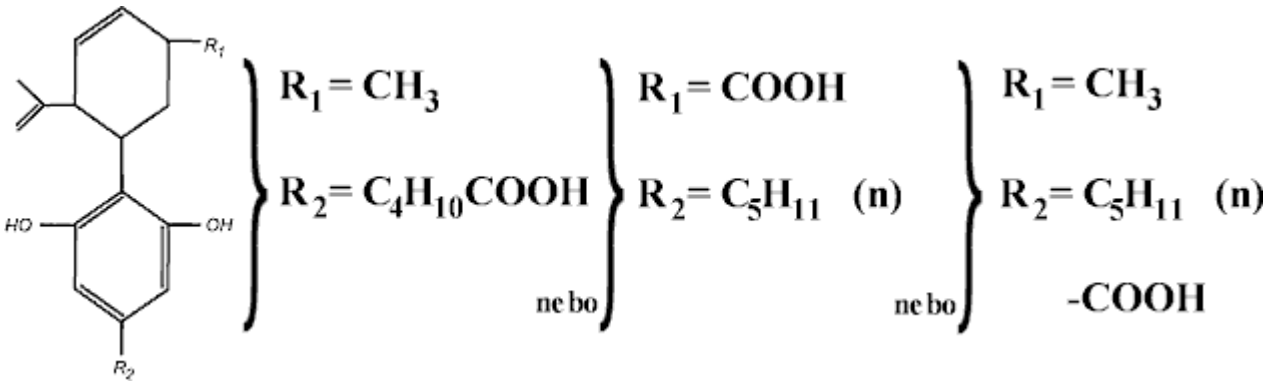

I. II. III.

The manufacturing process has been filed for patent.

Recently, attention has been paid again to the substances contained in the leaves of Cannabis sativa, var. indica, grown in our country, and one of us (Zd. K.) found considerable antibiotic properties of the raw ethyl alcohol extract against a number of pathogenic and non-pathogenic microorganisms. Its finding was confirmed in clinical practice by Soldán.

In our attempts to isolate this biologically active substance, we obtained in a crystalline state only an acid that was isolated as its diacetyl derivative with b.t. 80-100/127-128°, [ a ] 18 D = - 71° ± 4° (c = 0.671 in chloroform) with two hydrogenable double bonds. The analytical values obtained are best in agreement with the formula C 25H 32O 6 and less in agreement with the formula C 26H 34O 6. The extinction curve taken in the UF-region shows an inflection at 270 mμ (log e = 3.0 per mol weight of 428), which fully resembles the extinction curves of canabinnol derivatives.

According to these properties, the isolated acetyl acid derivative is most likely a homologous member of the previously isolated cannabinol (III), cannabidiol (I) or tetrahydrocannabinol (II) and belongs to one of the following forms:




The biologically active acid was called cannabidiolic acid.

Its cleavage into p-cymene and 1,3-dihydroxy-5-n-propionic acid, components that will determine the definitive summary formula, will be carried out after obtaining a larger amount of starting material.

After isolation of cannabidiolic acid, a neutral acetylated, probably phenolitic, not yet crystallizing residue remains, which also exhibits antibacterial properties.

In addition to this basic biologically active acid, we have succeeded in isolating another acid from the described material, which, however, is not biologically effective. The acid has a b. t. of 133° and the probable sum formula C 18H 16O 4. It is preliminarily designated as acid II. Finally, a substance was isolated from the neutral portion, which is probably alcohol with a b.t. of 59° and paraffin (b.t. 58°).

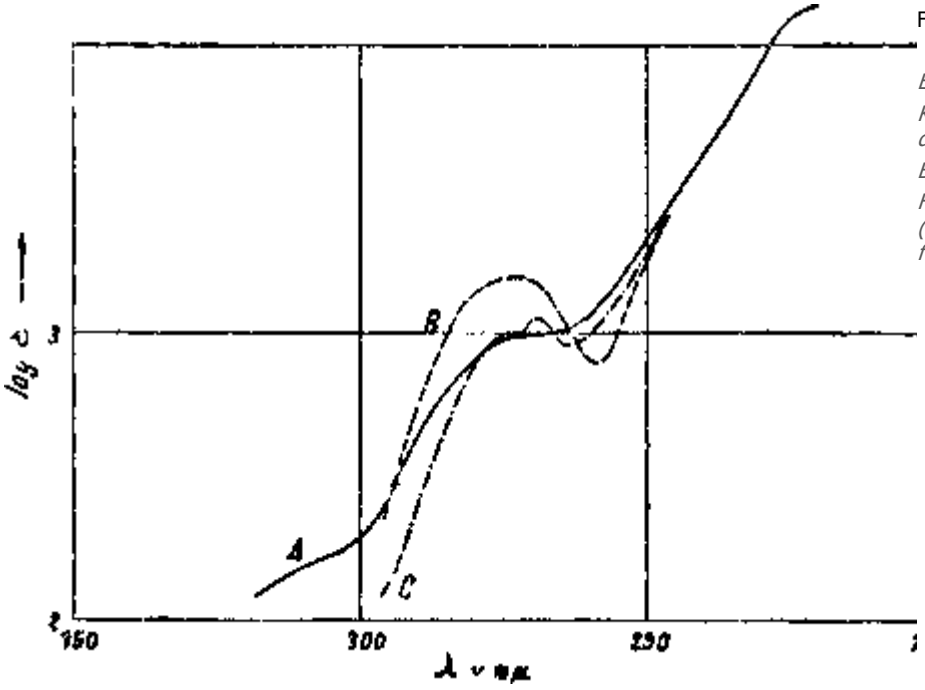



Fig. 1

*Extinction curve of acetyl acid derivative Kannabidiole (A) in UF-light. 4.91 mg of substance dissolved in 10 ml of ethanol. Beckmann spectrometer, DU model. For illustration, the curves of cannabinolin imethyl ether (B) and dihydrocannabinoldomethyl ether C are taken from the literature.*

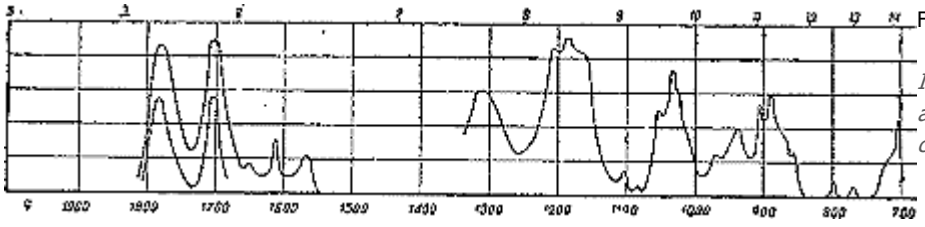


Fig. 2

*Infrared spectrum acetyl acid derivative cannabidiol in Nujol.*

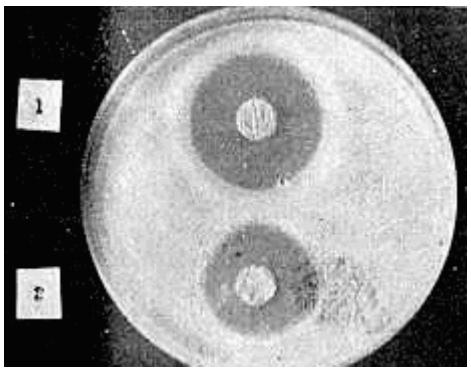



Fig. 3

*Comparison of inhibitory effects of acidic and phenolic IECi in vitro (masopeptone agar) on inoculated staphylococcus.*

*1. Acetylated and isolated acidic part from which acid crystallizes. cannabidiol. 2. Acetylated phenolic residue.*

## EXPERIMENTAL PART

The melting points were determined on the Kofler block and are corrected. Limit error up to 200° ± 2°, above 200° ± 3°. All distillations were carried out from a water bath with a reduced vacuum at 35°. Individual solutions were dried bezv. On 2SO 4.

### *Preparation of the extract*

**Attempt A.** 800 g of dried leaves of the drug Cannabis sativa, var. indica, grown by Prof. Dr. J. Kabelík in Velké Losiny in Moravia, altitude 450 m, a total of 17 l of pure alcohol was extracted. The obtained ethanolic extract was concentrated in a reduced vacuum from a 35° warm water bath. The obtained oily evaporator was dissolved in a mixture consisting of 1 liter of rain. water and 2 liters of petroleum ether, and transferred to the separating funnel. The aqueous part was washed successively with ether, chloroform and an ethanol-chloroform mixture (1:2). Biological tests (see table) have shown that antibacterial active substances are found only in the petroleum ether fraction, slightly in the etheric fraction.

| | **Proceeds** | **Biol. Activities** |
|---|---|---|
| The Petrolther Faction | 59 g | +++ |
| Etheric fraction | 0.6 g | + |
| Chloroform fraction | 0.2 g | - |
| Ethanol-chloroform fraction | 0.05 g | - |
| Aqueous solution | 6.3 g | - |

The petroleum etheric extract was further washed in separating funnels gradually by 200 ml of 2 % HCl, water, 2 % NaOH and again 3 times with water. Nothing could be isolated from the hydrochloric acid solution. However, a significant amount of the substance passed into the diluted lye, the basicity of which was carefully controlled during the experiment. The diluted solution of lye, into which acidic substances passed, was further purified by shaking it with petroleum ether. After acidification with dilute hydrochloric acid, a white villous substance, well soluble in ether, fell out of it. The yield of 7.88 g of brownish, glassy mass, which could not be crystallized from common solvents and which, as it turned out, is responsible for the antibacterial properties of the alcoholic extract from the leaves of cannabis.

*Attempt B.* 970 g of hemp leaves from 1952 were extracted with ethanol. After vacuum distillation of ethanol, the evaporator was mixed with 500 ml of 2% NaOH and 1 l of petroleum ether. The NaOH solution was then replaced with 200 ml of 2% NaOH and finally with the same amount of water. These alkaline-aqueous fractions were washed in additional separating funnels twice with 1 liter of petroleum ether each.

The first alkaline extract was brownish, clear, while the second solution contained a lot of microcrystalline substance, which was maintained at the interface between the water and the petroleum ether layer. This mass was therefore separated by centrifugation. These extracts were saturated with CO2 and shaken out with ether, into which only phenolithic substances were to pass. The aqueous residue, containing acids, was acidified and shaken out again by ether.

The sodium salt was decomposed by sulphuric acid, shaken out by ether, from which it easily crystallises and sublimated at 100 - 130°/1 mm Hg. The substance was designated as acid II and melts after sublimation and subsequent crystallization from ether and petroleum ether at 132 - 134°. The same substance is also obtained by extracting an ether solution, nas. Pom. CO2. The substance does not show any biological (antibacterial) activity.

| | | | |
|---|---|---|---|
| For C18H16O4 (296.31) calculated: C | 72.98 % H | 5.44% N.E. | 148,1 |
| found: C | 73.23 % H | 5.64% N.E. | 148,3 |
| | 73,35 | 5,68 | 148,0 |

*Attempt C.* 1,000 g of hemp leaves, collected in 1954 and also grown in Velké Losiny, were processed by the procedure described in experiment A and the same amount (1 kg) was extracted under cold benzene, in a perrolator, a total of 6 l. The benzene solution was concentrated in a reduced vacuum to a volume of 1 l, which was shaken out with a dilute solution of lye, which is supposed to provide biologically active acid after acidification.

*Attempt D.* 1 kg of hemp leaves from 1953 were extracted with petroleum ether and the petroleum ether extract was concentrated on a column. The rest was exaggerated by water vapour, with which a pleasantly scented essential oil passed, which had not yet been analysed. This essential oil could be concentrated by removing it into ether.

### *Treatment of neutral, biologically ineffective part*

Since it was not possible to obtain any crystalline substance from the neutral petroleum etheric residue by conventional chromatography, it was hydrolysed with alcoholic liquor in a nitrogen atmosphere and the alkaline solution was removed with ether. This neutral part was subjected to new chromatography for Al2O3 and this was passed with petroleum ether to a substance which, after recrystallization from petroleum ether, provided a b. t. 58°. With the ether, the substance left at b. t. 65°. The first substance is paraffin, the second substance is an as yet unidentified alcohol, which, however, does not show any antibacterial properties.

Alcohol analysis: C 73,85 %, H 12.01%, H 0.48%.

### *Processing of an etheric extract containing biologically active acidic components of cannabis*

Since we were unable to bring the above-described etheric acid extract (7.88 g) to crystallization from common solvents, the entire amount was acetylated with 50 ml of acetic anhydride and 3 g of anhydrous potassium acetate. After 2 hours of heating in a water bath, the unreactive anhydride was vacuum distilled and the residue after dissolution in ether was washed gradually with water, 5% soda solution and water again. In the etheric part purified in this way, 5.05 g of neutral matter remained, probably of a purely

phenolytic character before acetylation. This acetylated, originally phenolytic matter retains some of its antibacterial properties and its processing will be the subject of further work.

The ether-purified sodium solution was acidified with dilute sulfuric acid and the fallen white matter was removed into the ether. 3.1 g of yellowish glassy substance was obtained, which crystallizes by long-term standing. The substance crystallizes very well from a mixture of ethyl acetate and petroleum ether in colorless needles with a double melting point of 80-100/127-128°, [ and ] 18D = -71° ± 4° (c=0.671 in chloroform). The substance crystallized for the first time after six months of standing in the refrigerator at minus 3°. Further crystallization took place quickly after inoculation.

The substance is very well soluble in methanol, ethanol, ether, chloroform and dilute ammonia, from where it falls out after acidification as a white amorphous mass. It is well soluble in dilute Na2CO3, from where it also falls out after acidification. It is practically insoluble in water. With the end. Acid. Sulphur gives it a pink colour. Reaction with tetranitromethane positive.

| | | | | |
|---|---|---|---|---|
| Pro C25H32O6 (428,51) | | | | |
| Calculated: | C 70,07 | H 7.54 | - COCH3 20.1 | - COOH 10.75% |
| For C26H34O6 (442,53) | | | | |
| Calculated: | C 70,56 | H 7.74 | - COCH3 19,5 | - COOH 10,18 % |
| Found: | C 70,26 | H 7.69 | - COCH3 22.47 | - COOH 10.04% |
| | 70,17 | 7,58 | 21,85 | 9,96 % |
| | 70,01 | 7,55 | | |

Activated hydrogen found 0.35%. Sulfur, nitrogen, and methoxyl were not found.

Note on acetyl analyses: during saponification with sulphuric acid, subsequent dilution and restilation, the substance sublimated. Saponification was therefore carried out for 8 hours by boiling with alcoholic lye.

**Saponification of acetylated cannabidiolic acid**

300 mg of crystalline substance was dissolved in 5 ml of methanol, 1 ml of saturated methanolic NaOH was added and heated in a water bath under a refrigerant for 2 hours. Then the methanol was vacuum distilled, the rest dissolved in water (a poorly soluble sodium salt was formed), acidified and removed with ether. However, the substance could not be crystallized from common solvents.

### *Hydrogenation of the acetylated acid product. Cannabidiol*

401.3 mg of the acetylated compound described above was hydrogenated using 41.78 mg Adams-Shriner catalyst in methanol. Hydrogenation proceeded very quickly and was completed in practically 45 minutes. Hydrogen consumption (converted to VO equals 39.4 ml). The theory for 2 double bonds and C26H34O6 is equal to 40.6 ml.

The substance crystallizes from ether and petroleum ether in colourless needles b. t. 148-150°, [ a ] 18D = -22° ± 4° (c = 0.76 in chloroform).

| | | |
|---|---|---|
| Pro C25H36O6 (432,54) | | |
| Calculated: | C 69,42 % | H 8.39% |
| Pro C25H38O6 (434,55) | | |
| Calculated: | C 69,09 % | H 8.81% |
| Found: | C 70,05 % | H 8.67% |

The molecular weight, determined by Rast's method, gave a value of 430 ± 15.

*Effect of perphthalic acid on acetylderivative of acid. cannabidiol.*

500 mg acety, acid. Cannabidiolic acid was mixed with 1,500 mg of monoperphthalic acid in 100 ml of ether. After 36 hours of oxidation, the consumption of perphthalic acid was determined by titration, which corresponded to 2.46 F.

The oxidation product was further processed in the usual way, but it has not yet been possible to get it to crystallize from common solvents.

The analyses were carried out in the analytical laboratories of the Research Institute of Organic Synthesia in Pardubice-Rybitví (heads doc. Dr. J. Jureček and Dr. J. Večeřa), in the analytical department of the Institute of Organic Chemistry of the Czechoslovak Academy of Sciences in Prague and at Dr. Peisker in Bruck (Austria). In the institute of org. chemical Czechoslovak Academy of Sciences UF - and IR spectra were made. We thank everyone most sincerely.

## SUMMARY

It has been found that the antibacterial substances of cannabis leaves (Cannabis sativa L., indica variety) have an acidic character. Based on this, an acid was isolated in the form of its acetyl derivative, which has retained antibacterial properties against some bacterial strains. Acetyl acid derivative b. t. 80-100/127-128°, [ a ] 18D -71° ± 4° (in chloroform), probable sum formula C26H32O6 or C26H34O6 with two acetyl groups, two hydrogenable double bonds, one carboxyl group and one aromatic nucleus. This substance is similar to the previously isolated cannabidiol and was therefore called cannabidiolic acid. Especially hydrogenated substance is very easy to crystallize.

In addition to this biologically active acid, another acidic substance of b.t. 133° was isolated, but it is not biologically active, and finally paraffin was isolated

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