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Cannabis. XIV.[1] Two New Propyl Cannabinoids, Cannabicyclovarin and Δ^7-*cis*-Iso-tetrahydrocannabivarin, from Thai Cannabis

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Two new neutral propyl cannabinoids, cannabicyclovarin and Δ^7-*cis*-iso-tetrahydrocannabivarin have been isolated from Thai cannabis, "Meao strain." This is the first report of isolation of the latter, which has a novel skeleton, from natural sources.

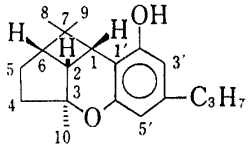
Keywords——Moraceae; Cannabis; propyl cannabinoid; cannabicyclovarin; Δ^7-*cis*-iso-tetrahydrocannabivarin; structure

As a continuation of our studies on cannabis, we have isolated new neutral propyl cannabinoids[2] and propyl cannabinoid acids[3] from Thai cannabis, 'Meao strain'. We now wish to describe the isolation and the structure elucidation of two new propyl cannabinoids from this Thai cannabis.

Cannabinoid acid fraction, which was obtained as described previously,[3] was decarboxylated and repeatedly purified by column chromatography on Silica gel and finally by preparative thin-layer chromatography (TLC) to give two new compounds, **1** and **2**, together with tetrahydrocannabivarin (THCV),[4] cannabichromevarin (CBCV),[2] tetrahydrocannabinol (THC), cannabichromene (CBC) and cannabivarin (CBV).[5]

The first compound, **1**, $C_{19}H_{26}O_2$, $[\alpha]_D$ 0°, colorless needles, mp 134—135.5°C, showed positive coloration with diazotized benzidine (red). The ultraviolet (UV) spectrum suggested the presence of a non-conjugated benzene ring (276, 284 nm). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum showed signals for one methyl group at δ 0.79 which was at abnormally high field compared with other cannabinoids, two methyl groups at δ 1.37, one benzyl methylene group at δ 2.44 as a triplet, one methine proton at δ 2.56 (dd, J=8 and 9 Hz), one benzyl methine proton at δ 3.04 (d, J=9 Hz), a hydroxyl proton at δ 4.40, and two aromatic protons at δ 6.17 and δ 6.32 (each d, J=2 Hz). This ^{1}H-NMR spectrum was similar to that of cannabicyclol (CBL)[6] except for the methylene region. The mass spectrum (MS) exhibited fragments, m/z 286 (M^+), 271, 204, 203 (100%) and 174. This fragmentation pattern is also the same as that of CBL,[7] except that all the masses are 28 units smaller. These data suggest that the new neutral propyl cannabinoid may be cannabicyclovarin (CBLV) and the structure

is shown as **1**. In order to confirm the structure, **1** was unequivocally identified by comparison with an authentic sample of CBLV obtained by irradiation[6] of CBCV. Although CBLV has been detected by gas liquid chromatography–MS(GC–MS),[8] this is the first time that CBLV has been isolated from natural sources.



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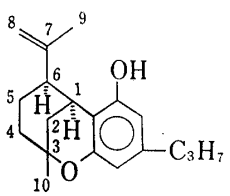
The second compound, **2**, $C_{19}H_{26}O_2$, $[\alpha]_D$ 0°, a colorless syrup, gave a red color with diazotized benzidine on TLC but showed less polarity than THCV. The MS showed the molecular ion at *m/z* 286 and fragment ions at *m/z* 271, 243, 203 (100%) and 174, suggesting **2** might be a neutral propyl cannabinoid. The UV and IR spectra give evidence that **2** has a non-conjugated benzene ring (275 and 282 nm) and a terminal methylene in the molecule (900 cm^{-1}). The ^{1}H-NMR spectrum shows signals for a side chain ω-methyl group at δ 0.92, one methyl group at δ 1.36, one vinylic methyl group at δ 1.78, benzyl methylene protons of the side chain at δ 2.48 (2H, t), one benzyl methine proton at δ 3.28 (q, J=3 and 6 Hz) and two aromatic protons at δ 6.18 and δ 6.27 (each d, J=1.5 Hz). The carbon (^{13}C)-NMR was useful for the establishment of several additional structure units. A typical singlet at 73.3 ppm is a quaternary carbon next to oxygen and one of two doublets is observed at 48.3 ppm, which is typical of a benzyl methine carbon. This pattern is observed in a tricyclic cannabinoid having a benzopyran ring in the molecule, such as THC.[9] **2** also showed five triplets due to methylene groups at 22.9, 24.0, 37.5, 37.7 and 39.5 ppm. The second and fourth signals can be assigned to β- and α-methylene groups of the side chain with the aid of literature data for THC.[9] Thus, three additional methylene groups should be present in the skeleton.

When the signal of the benzyl methine proton at δ 3.28 was irradiated, the multiplet centering at δ 1.86 changed into a triplet. On the other hand, the irradiation of the signal at δ 1.86 collapsed the benzyl methine proton at δ 3.28 to a doublet (J=3 Hz) and one proton of the terminal methylene at δ 4.86 into a doublet which may be caused by a long-range effect of the vinylic methyl group. Furthermore, when the broad peak near 2.3 ppm was irradiated, the benzyl methine proton at δ 3.28 changed into a broad triplet. These decoupling experiments (Fig. 1) were performed to gain information regarding the benzyl methine and vinylic proton environment. Furthermore, the coupling constant between C_1- and C_6–H (J=6 Hz) suggested that these vicinal protons should be *cis*, in good agreement with a consideration of the Dreiding mode of **2**. These data overall suggested the structure **2**.

In order to confirm the structure, **2** was directly compared with an authentic specimen (TLC, IR and ^{1}H-NMR) obtained from CBCV by Crombie's method[10] with some modification.

The pentyl homologue of **2** has been obtained synthetically from cannabichromene (CBC) by acid cyclization and called Δ^8-*cis*-iso-THC.[10–12] This is the first report of



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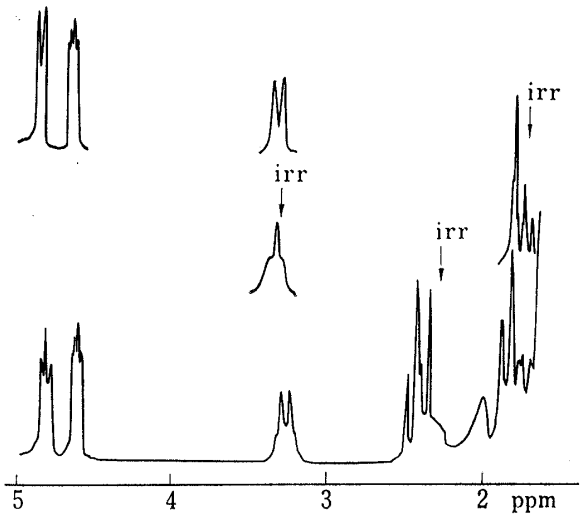

Fig. 1. ^{1}H-NMR Spectrum of Δ^7-*cis*-Iso-tetrahydrocannabivarin (**2**)

isolation of a cannabinoid having such a skeleton from natural sources, however since young Thai cannabis which had been stored was used in this study, **1** and **2** may be artefacts formed from cannabichromevarinic acid (CBCVA) or CBCV during storage.

Experimental

Melting points were taken on a Kofler block and are uncorrected. UV spectra were determined on a Hitachi 340 recording spectrophotometer. IR spectra were obtained with a Nihon Bunko Model DS-100 spectrometer. ^{1}H-NMR spectra were taken in $CDCl_3$ solution at 100 MHz on a JEOL PS-100 spectrometer; chemical shifts are given on the ppm scale with tetramethylsilane as an internal standard, and signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad). ^{13}C NMR spectra were taken in $CDCl_3$ solution at 25.05 MHz on a JEOL-FX 100 spectrometer. MS were taken on a JEOL D-300 machine. Optical rotations were taken with a JASCO DIP-4 digital polarimeter. Column chromatography was carried out with Kieselgel 60 (0.06—0.2 mm, Merck) using 50—100 times the quantity of the material.

Isolation of 1 and 2——Cannabinoid acid fraction (3.5 g), obtained from young Thai cannabis as described previously,[3] was decarboxylated by heating at 160°C for 15 min followed by column chromatography on Kieselgel 60 using benzene as a solvent to give THCV (800 mg) and CBCV (100 mg) in the pure state. THC (65 mg) was contaminated with cannabinol (CBN) and THCV but it was shown to be identical with an authentic specimen (TLC and GLC) without further purification. CBC (10 mg) was contaminated with trace amounts of CBCV but was shown to be identical with an authentic sample (TLC and GLC) without further purification. The first eluate, which contained two new phenolic compounds together with CBV, was repeatedly purified by preparative TLC to give **1** (5 mg) as colorless needles, and **2** (6 mg) as a colorless syrup.

1 (CBLV), colorless needles (from benzene–hexane), mp 134—135.5°C, $[\alpha]_D$ 0° ($c=0.25$, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 276 (3.12), 284 (3.11). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3600 (OH), 1622, 1578 (C=C). ^{1}H-NMR (in $CDCl_3$) δ: 0.79 (3H, s, C_8 or C_9–Me), 0.91 (3H, t, $J=7$ Hz, ω-Me), 1.37 (6H, s, C_{10} and C_8 or C_9–Me), 2.44 (2H, t, α-CH_2), 2.56 (1H, dd, $J=8$ and 9 Hz, C_2–H), 3.04 (1H, d, $J=9$ Hz, C_1–H), 4.40 (1H, OH), 6.17, 6.32 (each H, each d, $J=2$ Hz, aromatic H). MS m/z: 286 (M^+), 271, 204, 203 (100%), 174. Calcd for $C_{19}H_{26}O_2$: 286.1932. Found: 286.1929.

Synthesis of 1——CBCV (33 mg) was dissolved in *tert*-BuOH–acetone (1: 1; 5 ml) and irradiated with a high pressure mercury arc lamp (100 W) at 30°C for 7 h. After removal of the solvent by evaporation, the reaction mixture was purified by preparative TLC using benzene as a solvent to give **1**, mp 135°C. The spectral data (IR and ^{1}H-NMR) were identical with those of an authentic sample of the natural product, and the mixed mp was undepressed.

2(Δ^7-*cis*-iso-THCV), colorless syrup. $[\alpha]_D$ 0° ($c=0.31$, $CDCl_3$). UV λ_{max}^{MeOH} nm (log ε): 275 (3.10), 282 (3.12). IR $\nu_{max}^{CDCl_3}$ cm^{-1}: 3500 (OH), 1627, 1575 (C=C), 900 (terminal methylene). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, ω-Me), 1.36 (3H, s, C_3–Me), 1.78 (3H, s, C_7–Me), 1.86 (1H, m, C_6–H), 2.48 (2H, t, $J=8$ Hz, α-CH_2), 3.28 (1H, q, $J=3$ and 6 Hz, C_1–H), 4.56, 4.86 (1H × 2, each br d, C_8–CH_2), 6.18, 6.27 (1H × 2, each d, $J=1.5$ Hz). ^{13}C NMR ($CDCl_3$) ppm: 13.9 (q, C-ω), 22.4 (d, C-6), 22.9 (t, C-5)*, 24.0 (t, C-β), 28.8 (q, C-10), 32.9 (q, C-8), 37.5 (t, C-2)*, 37.7 (t, C-α), 39.5 (t, C-4)*, 48.3 (d, C-1), 73.7 (s, C-3), 107.1 (d, C-3′), 107.8 (d, C-5′), 111.3 (s, C-1′), 111.3 (t, C-9), 142.5 (s, C-4′), 149.6 (s, C-7), 153.2 (s, C-6′), 156.7 (s, C-2′) (*signals may be reversed). MS m/z: 286 (M^+), 271 (M−Me), 243, 203 (100%) and 174. Calcd for $C_{19}H_{26}O_2$: 286.1932. Found: 286.1934.

Synthesis of 2——CBCV (46 mg) in AcOH (1 ml) was refluxed for 2 h. After removal of AcOH by evaporation, the reaction mixture was purified by preparative TLC twice using benzene to give **2** (15 mg) as a syrup. The spectral data (IR and ^{1}H-NMR) were identical with those of an authentic sample of the natural product.

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Physiological and Biochemical Studies on Germinating Fungal Spores V.[1] Partial Purification and Characterization of Trehalase in Conidia of *Cochliobolus miyabeanus*

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Soluble trehalase (EC 3.2.1.28) was isolated and purified from the conidia of *Cochliobolus miyabeanus* by diethylaminoethyl Sephadex A-50 column chromatography and polyacrylamide gel electrophoresis. The molecular weight of this enzyme was estimated to be 160000 by the gel filtration method. Although the purified trehalase hydrolyzed both trehalose and maltose, this enzyme showed higher activity toward trehalose than maltose (the K_m values were 7.3×10^{-4} M for trehalose and 1.5×10^{-2} M for maltose). The optimal pH and temperature for the enzyme reaction were 4.0 and 37°C respectively. The enzyme became unstable at 50°C. This enzyme was competitively inhibited by fructose-6-phosphate and Na-pyruvate.

Keywords——trehalase; *Cochliobolus miyabeanus*; fungal spore; conidia; trehalose

In the previous paper,[1] we reported that trehalose stored in conidia of *Cochliobolus miyabeanus* was consumed rapidly within 15 min after the commencement of germination and that the trehalase activity which was detectable in the ungerminated conidia increased at that time, and then began to decrease gradually after 30 min of incubation. To clarify the regulatory mechanism of trehalase activity in the germinating conidia, we attempted the partial purification and characterization of the trehalase (EC 3.2.1.28) contained in the conidia.

Materials and Methods

Organism and Germinating Conditions——The harvesting procedure for conidia of *Cochliobolus miyabeanus* and the germinating conditions were as described previously.[1]

Extraction of Crude Trehalase——The conidia (10.0 g) were homogenized with 2 parts of carborundum in 0.01 M Tris-HCl buffer, pH 7.0, using a chilled pestle and mortar and the homogenate was made up to 200 ml with the same buffer and allowed to stand for 12 h. The cell debris was then removed by centrifugation (4000×g, 10 min), and the pH of the supernatant was adjusted to 3.5 by adding 1 M acetic acid. After removal of sediments by centrifugation (10000×g, 10 min), the same volume of chilled acetone was added to the supernatant and the mixture was allowed to stand for 6 h at 0°C. The precipitate was collected by centrifugation (10000×g, 15 min), resuspended in the same buffer (10 ml), and dialyzed against the same buffer (24 h, 4°C). After removal of some insoluble matter, the dialyzate was used as crude enzyme solution.

Enzyme Assay——The assay of glycosidase activity was based on the colorimetric determination of glucose released from the substrate. The reaction mixture consisted of the enzyme solution (0.1 ml), 0.1 M trehalose or an appropriate substrate (0.1 ml) and 0.1 M acetate buffer, pH 4.0, (0.3 ml). After incubation for 10 min at 37°C, glucose released was determined by using the Glucostat reagent (Worthington Biochemical Co.). Enzyme activity (unit) was expressed as μ mol of glucole released/min and the specific activity as units/mg of protein. Protein was determined by the method of Lowry *et al.*[2]

DEAE-Sephadex A-50 Column Chromatography——The crude enzyme solution (23 mg of protein) was applied to a diethylaminoethyl (DEAE)-Sephadex A-50 (acetate form) column (2.5×30 cm) preequilibrated