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Cannabis. X.[1] The Isolation and Structures of Four New Propyl Cannabinoid Acids, Tetrahydrocannabivarinic Acid, Cannabidivarinic Acid, Cannabichromevarinic Acid and Cannabigerovarinic Acid, from Thai Cannabis, 'Meao Variant'

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Four new cannabinoid acids, tetrahydrocannabivarinic acid, cannabidivarinic acid, cannabichromevarinic acid and cannabigerovarinic acid were isolated from Thai Cannabis, 'Meao variant' and these structures were elucidated on the basis of chemical and spectral data. This is the first example of the isolation of propyl cannabinoid acid from Cannabis.

Keywords——Moraceae; Cannabis; propyl cannabinoid acid; structure; biogenesis

Recently, many neutral propyl cannabinoids, cannabidivarin (CBDV, IIa),[3] tetrahydrocannabivarin (THCV, Ia),[4] cannabivarin (CBV),[5] cannabichromevarin (CBCV, IIIa)[1] and cannabigerovarin (CBGV, IVa),[1] have been isolated from the south Asian Cannabis. In the continuing investigation of the 'Meao' Cannabis, the seeds of which were collected in the Meao village of Thailand, by one of the authors in 1972, four new propyl cannabinoid acids are isolated and these structures are elucidated.

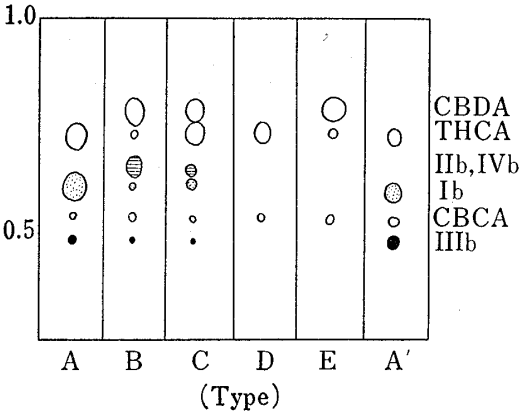
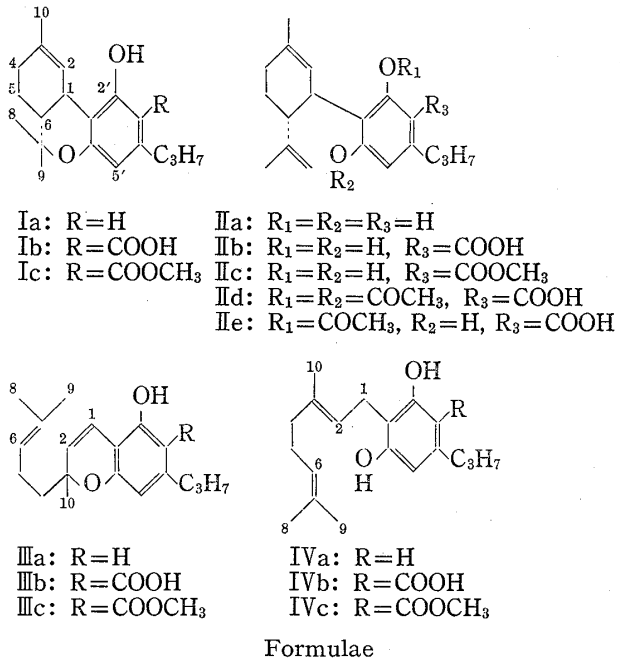


Fig. 1. Thin-Layer Chromatograms of Thai Cannabis, 'Meao Variant'

Sliicagel G.
Hexane-EtOAc(1: 2).
A' : young plant of type A.



Formulae

1) Part IX: Y. Shoyama, H. Hirano, M. Oda, T. Somehara and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **23**, 1894 (1975).
2) Location: *3-1-1 Maidashi, Higashiku, Fukuoka.*
3) L. Vollner, D. Bieniek and F. Korte, *Tetrahedron Lett.*, **1969**, 145.
4) E.W. Gill, *J. Chem. Soc.*, **1971**, 579.
5) F.W.H.M. Merkus, *Nature*, **232**, 579 (1971).

A survey of the 'Meao' Cannabis showed that the plants of the strain had various types of cannabinoid pattern as described by thin-layer chromatogtam (TLC) (Fig. 1). For convenience we named these new compounds, Ib, IIb, IIIb and IVb. The amount of IIIb seemed to be somewhat larger in vegetative phase than in reproductive period.

The benzene extractives of dried leaves were treated with acetone and chromatographed on a polyamide to give the cannabinoid acid fraction as done previously.[6] The fraction was further purified by column chromatography over silica gel and polyamide. Finally, Ib, IIb, IIIb and IVb were isolated in pure state.

Compound Ib, $C_{20}H_{26}O_4$, $[\alpha]_D$ −188° (in $CHCl_3$), was isolated from type A as a major component. Ib was colorless syrup and showed a red coloration with the addition of diazotized benzidine reagent. Ultraviolet(UV) spectrum of Ib showed maxima at 224 nm, 262 nm and 303 nm. Infrared(IR) spectrum showed the presence of hydroxyl(3400 cm^{-1}) and the chelated carboxyl group(1655 cm^{-1}). These findings indicated that Ib should be cannabinoid acid. In the nuclear magnetic resonance (NMR) spectrum of Ib, a broad singlet at 6.40 ppm and a singlet at 6.22 ppm were assigned to the vinyl proton and the aromatic proton, respectively. A chelated hydroxyl proton appeared at 12.18 ppm. This NMR spectrum is similar to that given by tetrahydrocannabinolic acid(THCA),[7] differing only in the regions of the methylene at 2.0—2.2 ppm and the aromatic proton at 6.22 ppm. Ib was esterified by usual manner to give methyl ester, Ic, $C_{21}H_{28}O_4$, $[\alpha]_D$ −153° (in $CHCl_3$), mass (MS) spectrum *m/e*: 344(M^+), 312, 297, 278, 269, 256, 223 and 205. This MS spectrum has the characteristic fragmentation pattern of THCA methyl ester, the difference being that all masses are 28 units smaller (the difference between the mass of C_5H_{11} and C_3H_7). Upon decarboxylation of Ib with heating at 150—160° for 10 min[6] followed by purification with preparative TLC, colorless syrup, Ia was obtained. Ia was identified with THCV by the direct comparison ($[\alpha]_D$, UV, IR, NMR and MS) with an authentic sample.

All of the properties of Ib mentioned above strongly suggest that Ib must be identical with the primary cannabinoid acid of THCV, tetrahydrocannabivarinic acid (THCVA) and the structure has been established as Ib.

Compound IIb, $C_{20}H_{26}O_4$, mp 102—105°, optical rotatory dispersion (ORD) (*c*=0.1, MeOH) [θ](nm): −1650(315), −627(400), −198(500), was isolated from type B as a major component. IIb was in the form of colorless needles and reacting positively to the Beam reagent(violet) and the diazotized benzidine reagent (orange). The UV spectrum of IIb showed maxima at 224 nm, 263 nm and 303 nm. The IR spectrum showed the presence of hydroxyl(3400 cm^{-1}), carboxyl(1690 cm^{-1}) and terminal methylene(890 cm^{-1}) groups. The NMR spectrum of IIb revealed the presence of the terminal methylene protons(4.40 and 4.55 ppm, J=14 Hz), vinyl proton (5.56 ppm), aromatic proton(6.23 ppm), hydroxyl proton (6.62 ppm) and the chelated hydroxyl proton(11.81 ppm) in the structure. IIb was methylated the usual manner to give methyl ester, IIc, $C_{21}H_{28}O_4$. The NMR spectral signal of carbomethoxyl(3.88 ppm) appeared in addition to the above signals and these coincided with those given by cannabidiolic acid (CBDA) methyl ester.[8] The MS spectrum of IIc gave a fragment pattern similar to that of CBDA methyl ester with the decrease of 28 units. On acetylation in general way, IIb gave a diacetate, IId, as colorless needles, mp 102—106°, $[\alpha]_D$ −78° which showed no hydroxyl absorption band in the IR spectrum. NMR spectrum of the acetate indicated two acetyl protons at 2.22 ppm. The mild saponification of IId with methanolic $NaHCO_3$ reverted IIe, monoacetate of IIb, mp 116—118°. $[\alpha]_D$−2.6° which had one hydroxyl group at 6.33 ppm ascribable to $C_{6'}$–OH and IIb. Upon decarboxylation IIb gave a neutral

6) Y. Shoyama, R. Oku, T. Yamauchi and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1927 (1972).
7) T. Yamauchi, Y. Shoyama, H. Aramaki, T. Azuma and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1075 (1967).
8) R. Mechoulam and Y. Gaoni, *Tetrahedron*, **21**, 1223 (1965).

cannabinoid, IIa, $C_{19}H_{26}O_2$, mp 115—118°, which was identified by the comparison (UV, IR and NMR) with CBDV.[3] By analogy with THCVA, IIb can be regarded as a propyl homologue of CBDA, cannabidivarinic acid(CBDVA), and its structure can be formulated as IIb.

Furthermore, the structures of Ib and IIb are confirmed by the chemical correlation concerning Δ^3-tetrahydrocannabivarinic acid (Δ^3-THCVA) and cannabivarinic acid(CBVA) as indicated in Chart 1 as has been established in the case of pentyl homologues.[9]

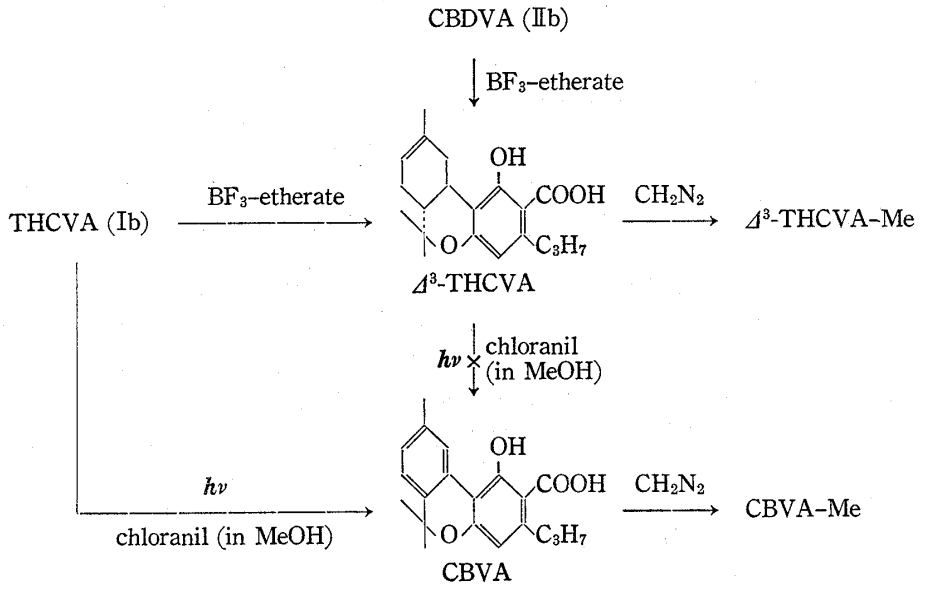


Chart 1. The Chemical Relation of THCVA and CBDVA

Compound IIIb, $C_{20}H_{26}O_4$, $[\alpha]_D$—4.8°(in $CHCl_3$), was isolated from younger Cannabis of type A as a minor component. IIIb was a colorless syrup showing a brownish red colorlation with the addition of diazotized benzidine reagent and typical blue white fluorescent spot when exposed under UV lamp. UV spectrum of IIIb had maxima at 249 nm, 255 nm, 290 nm and 336 nm and IR spectrum showed the presence of hydroxyl(3400 cm^{-1}) and the chelated carboxyl group(1660 cm^{-1}). In the NMR spectrum of IIIb, a pair of doublet at 5.45 ppm and 6.73 ppm(J=10 Hz) were assigned to the vinyl protons of chromene moiety, a triplet at 5.08 ppm was assigned to the another vinyl proton, and the chelated hydroxyl proton was found in the general region(11.86 ppm). All of the above data strongly suggest that IIIb might be a primary cannabinoid acid of CBCV.[1] It is similar to cannabichromenic acid (CBCA)[10] and essentially identical to that of CBCV,[1] except for the presence of only one aromatic proton(6.21 ppm) on the divarinol moiety. In order to confirm the structure of IIIb, IIIb was treated with diazomethane to give IIIc, $C_{21}H_{28}O_4$, which showed a new singlet at 3.91 ppm which was assigned to carbomethoxyl group. MS spectrum of IIIc exhibited fragment ions, molecular ion m/e 344, 329(M^+-CH_3), 313(M^+-OCH_3), 262, 261, 230 and 229 indicating that IIIc must be cannabichromevarinic acid(CBCVA) methyl ester. Furthermore, the structure of IIIb was elucidated by the decarboxylation of IIIb followed to give CBCV[1] which was identified by the direct comparison with an authentic sample. Finally, the structure was confirmed by the comparison with synthetic CBCVA.[11]

Compound IVb, $C_{20}H_{28}O_4$, mp 66—68°, was isolated from type A as a minor component. IVb was colorless needles and gave positive reactions to the Beam test(violet) and the diazotized benzidine reagent (orange). The UV spectrum of IVb showed maxima at 255 nm and 300 nm.

9) Y. Shoyama, T. Yamauchi and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **18**, 1327 (1970).
10) Y. Shoyama, T. Fujita, T. Yamauchi and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1157 (1968).
11) The detail will be reported elsewhere.

The IR spectrum showed a hydroxyl group (3400 cm^{-1}) and the chelated carboxyl group (1650 cm^{-1}). The NMR spectrum of IVb showed signals for three methyls on the vinyl position at 1.60, 1.68 and 1.82 ppm, one benzyl methylene at 3.44 ppm(doublet, $J=6$ Hz) and one aromatic proton at 6.27 ppm. These spectral data suggest that IVb might be the primary cannabinoid acid of CBGV, cannabigerovarinic acid(CBGVA). CBGVA was further assigned structure IVb on the basis of its conversion, through decarboxylation to CBGV[1] of which structure had been assumed to be IVa and finally the structure has been unequivocally established by the comparison with synthetic CBGVA.[12]

The present paper is the first example of the isolation of propyl cannabinoid acid from Cannabis. Although further occurrence of cannabigerovarinic acid monomethyl ether (CBG VAM) was expected, in spite of the detailed survey of this strain, no methylated cannabinoid was observed.

It now seems clear that the primary propyl cannabinoid acids, CBGVA, CBDVA, THCVA and CBCVA are biosynthesized by the acetate-malonate-mevalonate pathway like the pentyl homologues.[14]

The studies on the biosynthesis and the breeding of the strain are in progress.

Experimental

Melting points were taken on a Kofler block and are uncorrected. UV spectra were determined by Hitachi 124 Spectrophotometer. IR spectra were obtained with a Nihon Bunko Model DS-301 Spectrophotometer. NMR spectra were taken in $CDCl_3$ solution at 100 MHz on a JEOL PS-100 Spectrometer and chemical shift were given in (ppm) scale with tetramethyl silane as internal standards, signal multiplicaties were represented by s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). MS spectra were taken on a JEOL-JMS-OISG. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. ORD spectra were recorded with a JASCO ORD/UV-5 recording spectropolarimeter. Gas liquid chromatography (GLC) was conducted under the following conditions: Shimadzu Gas Chromato GC-4BM with 1.5% OV-1 (2 m×3 mm), column temperature 220°, detector temperature 260°, carrier gas; N_2 40 ml/min, H_2 80 ml/min, air 500 ml/min. Thin-layer plate was prepared with Kieselgel G (Merck) and performed with the following solvent systems: hexane–EtOAc (1: 2), $CHCl_3$–MeOH–H_2O (30: 10: 1), benzene–MeOH–AcOH (45: 8: 4) (for cannabinoid acid); benzene, hexane–benzene–diethylamine (20: 10: 1) (for neutral cannabinoid); hexane–$CHCl_3$–EtOAc (40: 2: 1) (for cannabinoid acid methyl ester). Column chromatography was carried out with Kieselgel 60 (0.06—0.2 mm, Merck) in 50—150 times quantity of the material.

Table 1. *Rf* Value, Color and Relative Retention Time of the Cannabinoids

Compd.	*Rf* value(TLC)[a]		Color[b]		Relative t_R (GLC)
	I	II	I	II	
CBDV		0.37	Orange	Violet	1.00(4.29 min)
CBDVA	0.67		Orange	Violet	1.00
THCV		0.30	Red		1.30
THCVA	0.63		Red		1.30
CBCV		0.26	Brownish red		1.00
CBCVA	0.52		Brownish red		1.00
CBGV		0.21	Orange	Violet	1.56
CBGVA	0.63		Orange	Violet	1.56
Δ^3-THCV		0.30	Red		1.22
Δ^3-THCVA	0.64		Red		1.22
CBV		0.28	Reddish violet		1.56
CBVA	0.44		Reddish violet		1.56

a) Solvent I : hexane–EtOAc (1: 2), II : benzene.
b) Spot test I : diazotized benzidine reagent, II : beam reagent.

12) CBGVA was synthesized by a procedure of Mechoulam and his coworkers[13] with modification and the detail was given in the experimental section.
13) R. Mechoulam and Z. Ben-Zvi, *Chem. Commun.*, **1969**, 343.
14) Y. Shoyama, M. Yagi and I. Nishioka, *Phytochemistry*, **14**, 2189 (1975).

Extraction and Separation of Cannabinoid Acid Fraction——Air dried leaves were extracted with benzene twice. The extractive was treated with cold acetone and the insoluble portion was removed by filtration. The filtrate was evaporated and then the residue was chromatographed on a polyamide in 2—5 times quantity of the material eluting with H_2O–MeOH (1: 1—1: 6) to give cannabinoid acid fraction as described previously.[6]

Decarboxylation of Cannabinoid Acid——Cannabinoid acid (approximately 15—20 mg) was decarboxylated by heating at 150—160° for 10 min to give neutral cannabinoid which was further purified by column chromatography on silica gel or preparative TLC on silica gel plate using benzene as a developing solvent if necessary.

Methylation of Cannabinoid Acid——Cannabinoid acid (approximately 15—20 mg) was treated with ether solution of diazomethane at 5°. The crude product was passed through a silica gel column eluting with hexane–EtOAc (60: 1) to give cannabinoid acid methyl ester.

Isolation of Ib (THCVA)——Cannabinoid acid fraction (12 g) obtained from the dried leaves (850 g, type A) was repeatedly column chromatographed over silica gel to give Ib (65 mg), colorless syrup, $[\alpha]_D^{17}$ −188° (c=0.33, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 224 (4.51), 262 (3.99), 303 (3.70). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1655 (C=O), 1620, 1568 (C=C). NMR (in $CDCl_3$) δ: 0.98 (3H, t, J=7 Hz, ω-CH_3), 1.10, 1.23, 1.44 (3H×3, each s, $C_{8,9,10}$-CH_3), 2.00—2.20 (2H, m, β-CH_2), 2.68—2.90 (2H, m, α-CH_2), 3.10—3.30 (2H, broad d, C_1–H), 6.22 (1H, s, C_5'–H), 6.40 (1H, broad s, C_2–H), 12.18 (1H, s, C_2'–OH). *Anal.* Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.83; H, 8.01. Ib was decarboxylated to give Ia which was identified with an authentic sample (UV, IR, NMR and MS).

Ic (THCVA Methyl Ester)——Colorless syrup, $[\alpha]_D^{20}$ −153° (c=0.65, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 225 (4.31), 274 (4.09), 308 (3.69). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1730, 1640 (C=O), 1615, 1568 (C=C). MS m/e: 344 (M^+), 312, 297, 278, 269, 256, 223, 205, Calcd. for $C_{21}H_{28}O_4$: 344.199. Found: 344.195.

Isolation of IIb (CBDVA)——Cannabinoid acid fraction (4.5 g) which was obtained from the dried leaves (1.15 kg, type B) was repeatedly column chromatographed on polyamide eluting with H_2O–MeOH (1: 3—1: 4) and further purified by silica gel column chromatography. Finally recrystallized from hexane–$CHCl_3$ to give IIb (213 mg), colorless needles, mp 102—105°, ORD (c=0.1, MeOH) $[\theta]^{22}$ (nm): −1650 (315), −627 (400), −198 (500), UV λ_{max}^{MeOH} nm (log ε): 224 (4.46), 263 (3.91), 303 (3.60). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1690 (C=O), 1620, 1580 (C=C), 890 (>C=CH_2). NMR (in $CDCl_3$) δ: 0.92 (3H, t, J=7 Hz, ω-CH_3), 1.71, 1.78 (3H×2, each s, $C_{8,9}$-CH_3), 2.88 (2H, m, α-CH_2), 4.10 (1H, broad s, C_1–H), 4.40, 4.55 (2H, d, J=14 Hz, >C=CH_2), 5.56 (1H, s, C_2–H), 6.23 (1H, s, C_5'–H), 6.62 (1H, s, C_6'–OH), 11.81 (1H, s, C_2'–OH). *Anal.* Calcd. for $C_{20}H_{26}O_4$: C, 72.70; H, 7.93. Found: C, 72.62; H, 7.88. IIb was decarboxylated to give IIa which was identified by the direct comparison (mp, UV, IR, NMR and MS) with an authentic sample.

IIc (CBDVA Methyl Ester)——Colorless syrup, $[\alpha]_D^{14}$ +16° (c=0.55, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 224 (4.64), 273 (4.33), 309 (3.90). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725, 1645 (C=O), 1620, 1580 (C=C), 890 (>C=CH_2). NMR (in $CDCl_3$) δ: 0.90 (3H, t, J=7 Hz, ω-CH_3), 1.70, 1.78 (3H×2, each s, $C_{8,9}$-CH_3), 2.78 (2H, q, J=3 Hz, 7 Hz, α-CH_2), 3.88 (3H, s, $COOCH_3$), 4.10 (1H, broad s, C_1–H), 4.38, 4.50 (2H, broad d, J=13 Hz, >C=CH_2), 5.54 (1H, broad s, C_2–H), 6.21 (1H, s, C_5'–H), 6.46 (1H, s, C_6'–OH), 11.96 (1H, s, C_2'–OH). MS m/e: 344 (M^+), 312, 297, 259, 217. Calcd. for $C_{21}H_{28}O_4$: 344.199. Found: 344.195.

Acetylation of IIb——IIb (100 mg) was acetylated with Ac_2O–pyridine (1: 1) at room temperature to give crude IId which was passed through a silica gel column eluting benzene–acetone (1: 2) to give IId, colorless needles, mp 102—106°, $[\alpha]_D^{17}$ −78° (c=0.3, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 275 (3.06), 310 (2.83). IR ν_{max}^{KBr} cm^{-1}: 1770, 1750, 1705 (C=O), 1615, 1572 (C=C), 895 (>C=CH_2). NMR (in $CDCl_3$) δ: 0.92 (3H, t, J=7 Hz, ω-CH_3), 1.59, 1.68 (3H×2, each s, $C_{8,9}$-CH_3), 2.22 (3H×2, s, OAc), 2.76 (2H, m, α-CH_2), 3.58 (1H, m, C_1–H), 4.48, 4.57 (2H, d, J=10 Hz, >C=CH_2), 5.22 (1H, s, C_2–H), 6.85 (1H, s, C_5'–H). *Anal.* Calcd. for $C_{24}H_{30}O_6$: C, 69.54; H, 7.30. Found: C, 69.24; H, 7.34.

Saponification of IId——IId (50 mg) was added to the solution of MeOH (4 ml) and $NaHCO_3$ (100 mg) in H_2O (3 ml) and left stand at room temperature for 15 hr. The reaction mixture was acidified with 10% H_2SO_4 and extracted with ether, washed and then dried. The crude mixture (50 mg) was purified by column chromatography on silica gel using hexane–EtOAc (5: 1) to (3: 1). Fraction 4—11 were pooled and the solvent was evaporated to give pure IIb (13 mg). Fraction 23—27; II_e (8 mg) was recrystallizatd from hexane–$CHCl_3$ mixture to give colorless needles, mp 116—118°, $[\alpha]_D^{18}$ −2.6° (c=0.40, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1740 (OAc), 1690 (COOH), 1610, 1580 (C=C), 890 (>C=CH_2). NMR (in $CDCl_3$) δ: 0.92 (3H, t, J=7 Hz, ω-CH_3), 1.63, 1.80 (3H×2, each s, $C_{8,9}$-CH_3), 2.24 (3H, s, C_2'-OAc), 2.74 (2H, m, α-CH_2), 3.50 (1H, broad s, C_1–H), 4.42, 4.61 (2H, d, J=17 Hz, >C=CH_2), 5.54 (1H, broad s, C_2–H), 6.33 (1H, broad s, C_6'–OH), 6.64 (1H, s, C_5'–H).

Isomerization of Ib and IIb to Δ^3-THCVA——Ib (100 mg) was dissolved in dioxane (6 ml) and then BF_3-ether solution (0.5 ml) was added. The mixture was left stand at room temperature for 4 hr. The reaction mixture was diluted with H_2O and extracted with ether 3 times. Ether layer was washed with H_2O and then dried over Na_2SO_4. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel using hexane–EtOAc (5: 1) as a solvent to give colorless syrup (Δ^3-THCVA) (80 mg), $[\alpha]_D^{26}$ −268° (c=1.09, $CHCl_3$), UV λ_{max}^{MeOH} nm (log ε): 223 (4.92), 263 (4.11), 302 (3.81). NMR (in $CDCl_3$) δ: 0.97 (3H, t, J=7 Hz, ω-CH_3), 1.10, 1.40, 1.70 (3H×3, each s, $C_{8,9,10}$-CH_3), 2.60—3.00 (2H, m, α-CH_2),

3.36 (1H, dd, J=6 Hz, 18 Hz, C_1–H), 5.40 (1H, broad s, C_4–H), 6.25 (1H, s, $C_{5'}$–H), 12.07 (1H, s, $C_{2'}$–OH). II_b (50 mg) was worked up in the same way as I_b to give Δ^8-THCVA (45 mg), which was identified by the direct comparison ($[\alpha]_D$, UV and NMR) with an authentic sample.

Δ^8-THCVA Methyl Ester——Δ^8-THCVA was methylated to give Δ^8-THCVA methyl ester, colorless syrup, $[\alpha]_D^{23}$ −224° (c=0.25, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 223 (4.34), 271 (4.04), 305 (3.53). IR ν_{max}^{KBr} cm^{-1}: 3700 (OH), 1650 (C=O), 1620, 1575 (C=C). NMR (in $CDCl_3$) δ: 0.95 (3H, t, J=7 Hz, ω-CH_3), 1.11, 1.39, 1.74 (3H×3, each s, $C_{8,9,10}$–CH_3), 2.70—2.90 (2H, m, α-CH_2), 3.37 (1H, dd, J=6 Hz, 14 Hz, C_1–H), 3.93 (3H, s, $COOCH_3$), 5.40 (1H, broad s, C_4–H), 6.20 (1H, s, $C_{5'}$–H), 12.26 (1H, s, $C_{2'}$–OH). MS m/e: 344 (M^+), 313, 312, 297, 269, 261, 256, 244, 229.

Conversion of Ib to CBVA——The solution of Ib (180 mg) and chloranil (100 mg) in MeOH (100 ml) was irradiated at room temperature with UV light for 50 hr as described previously.[9] The reactant was purified by column chromatography on silica gel (28 g) using hexane–EtOAc (4: 1) to (1: 1) as a solvent to give CBVA (107 mg), from (1: 1) eluate, which was then recrystallized from hexane–$CHCl_3$ to give colorless prisms, mp 129—133°. UV λ_{max}^{MeOH} nm (log ε): 232 (4.30), 256 (4.36), 290 (3.99), 322 (3.74). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1640 (C=O), 1620, 1565 (C=C), 870. NMR (in $CDCl_3$) δ: 0.99 (3H, t, J=7 Hz, ω-CH_3), 1.63 (6H, s, $C_{8,9}$–CH_3), 2.41 (3H, s, C_{10}–CH_3), 2.97 (2H, t, J=8 Hz, α-CH_2), 6.42 (1H, s, $C_{5'}$–H), 7.10 (2H, s, $C_{4,5}$–H), 8.46 (1H, s, C_2–H), 12.74 (1H, s, $C_{2'}$–OH).

CBVA Methyl Ester——CBVA was methylated to give CBVA methyl ester which was then recrystallized from MeOH to give colorless prisms, mp 104—107°. UV λ_{max}^{MeOH} nm (log ε): 233 (4.12), 266 (4.56), 328 (3.68). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH), 1730, 1640 (C=O), 1615, 1580 (C=C), 870. NMR (in $CDCl_3$) δ: 0.98 (3H, t, J=7 Hz, ω-CH_3), 1.63 (3H×2, s, $C_{8,9}$–CH_3), 2.43 (3H, s, C_{10}–CH_3), 2.88 (2H, t, J=8 Hz, α-CH_2), 3.98 (3H, s, $COOCH_3$), 6.40 (1H, s, $C_{5'}$–H), 7.12 (2H, s, $C_{4,5}$–H), 8.46 (1H, s, C_2–H), 12.38 (1H, s, $C_{2'}$–H). MS m/e: 340 (M^+), 325, 308, 293, 263. Calcd. for $C_{21}H_{24}O_4$: 340.167. Found: 340.169.

Isolation of IIIb——Cannabinoid acid fraction (71 g) which was obtained from the dried leaves (10.4 kg, young Cannabis of type A; 3 months old) was column chromatographed on silica gel repeatedly to give IIIb (96 mg) as a colorless syrup, $[\alpha]_D^{17}$ −4.8°, UV λ_{max}^{MeOH} nm (log ε): 249 (4.28), 255 (4.23), 290 (3.43), 336 (2.95). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3240 (OH), 1730, 1720, 1660 (C=O), NMR (in $CDCl_3$) δ: 0.94 (3H, t, J=7 Hz, ω-CH_3), 1.39, 1.56, 1.65 (3H×3, each s, $C_{8,9,10}$–CH_3), 2.89 (2H, t, J=8 Hz, α-CH_2), 5.08 (1H, t, J=8 Hz, C_6–H), 5.45 (1H, d, J=10 Hz, C_2–H), 6.21 (1H, s, $C_{5'}$–H), 6.73 (1H, d, J=10 Hz, C_1–H), 11.86 (1H, s, $C_{2'}$–OH). IIIb was decarboxylated to give III_a which was identified by the direct comparison (UV, IR, NMR and MS) with an authentic sample.

IIIc (CBCVA Methyl Ester)——Colorless syrup, UV λ_{max}^{MeOH} nm (log ε): 254 (4.44), 261 (4.45), 279 (3.79), 326 (3.44). IR ν_{max}^{KBr} cm^{-1}: 3200 (OH), 1650 (C=O). NMR (in $CDCl_3$) δ: 0.96 (3H, t, J=7 Hz, ω-CH_3), 1.40, 1.59, 1,67 (3H×3, each s, $C_{8,9,10}$–CH_3), 2.81 (2H, t, J=8 Hz, α-CH_2), 3.91 (3H, s, $C_{3'}$–$COOCH_3$), 5.45 (1H, d, J=10 Hz, C_2–H), 6.18 (1H, s, $C_{5'}$–H), 6.72 (1H, d, J=10 Hz, C_1–H), 11.90 (1H, s, $C_{2'}$–H). MS m/e: 344 (M^+), 329, 313, 262, 261, 230, 229. Calcd. for $C_{21}H_{28}O_4$: 344.201. Found: 344.199.

Isolation of IVb (CBGVA)——Cannabinoid acid fraction (111 g) which was obtained from the dried leaves (7.5 kg, type A) was purified by column chromatography on silica gel eluting with hexane–EtOAc and benzene–acetone (5: 1) repeatedly and then recrystallized from hexane–$CHCl_3$ to give colorless needles (24 mg), mp 66—68°, UV λ_{max}^{MeOH} nm (log ε): 255 (3.66), 300 (3.28). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3400 (OH), 1650 (C=O), 1580 (C=C). NMR (in $CDCl_3$) δ: 0.96 (3H, t, J=7 Hz, ω-CH_3), 1.60, 1.68, 1.82 (3H×3, each s, $C_{8,9,10}$–CH_3), 2.90 (2H, t, J=6 Hz, α-CH_2), 3.44 (2H, d, J=6 Hz, C_1–CH_2), 4.80—5.40 (3H, m, $C_{2,6}$–H and OH), 6.27 (1H, s, $C_{5'}$–H). IVb was decarboxylated to give IVa which was identified with an authentic sample by TLC and GLC.

Synthesis of IVb——Mg (56.4 mg) was refluxed in absolute MeOH (4.5 ml) for approximately 30 min. MeOH was completely evaporated *in vacuo*. CO_2 liberated from $BaCO_3$ (590 mg) was absorbed in the above magnesium methylate in DMF (0.5 ml) at −10° for 1 hr. Dried CBGV (156 mg) was added to the methylmagnesium carbonate–DMF solution and then heated at 120° for 1 hr. After cooling the reaction mixture was adjusted at pH 2.0 with dil. HCl and extracted with 10 ml of $CHCl_3$–MeOH (2: 1). The organic fraction was washed according to Folch's method.[15] The crude product was purified on silica gel (20 mg) using hexane–EtOAc (1: 1) as a solvent, Fr. 2—5; CBGV (110 mg), Fr. 8—9; IV (30 mg) was crystallized from hexane–$CHCl_3$ to give colorless needles, mp 66—68°. MS: Calcd. for $C_{20}H_{28}O_4$: 332.199. Found: 332.194. and identified by the direct comparison (mixed mp, UV, IR, and NMR) with an authentic sample. Synthetic IV_b was decarboxylated back to IV_a which was identified by the direct comparison (TLC and GLC) with an authentic sample.

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