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Cannabis. V.[1] Cannabigerolic Acid Monomethyl Ether and Cannabinolic Acid

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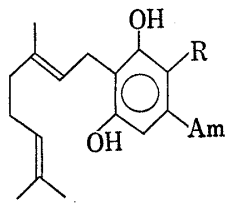
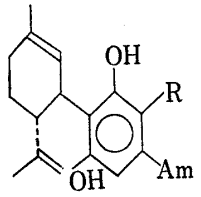
The original cannabinoids including Δ^2-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), cannabichromenic acid (CBCA), and a new component, cannabigerolic acid monomethyl ether (CBGAM) were isolated with simple column chromatographic technique from the fresh Cannabis of the domestic strain.

Cannabinolic acid (CBNA), observed in the stored Cannabis, was synthesized from THCA by means of ultraviolet (UV) light irradiation, and artificial conditions to yield CBNA from THCA, comparing with Δ^3-isomer, were investigated.

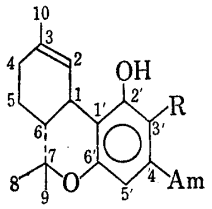
Among the phenol carboxylic acids of Cannabis (hemp, marihuana, hashish), cannabidiolic acid (CBDA) was first obtained by Šantavy's group[3] and by Schultz, *et al.*[4] Mechoulam and Gaoni reported the alumina chromatographic fractionation of cannabigerolic acid (CBGA), CBDA and cannabinolic acid (CBNA), as their methyl esters,[5] the regeneration to original



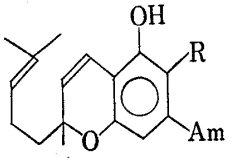
cannabigerolic acid (CBGA) : R=COOH
cannabigerol (CBG) : R=H



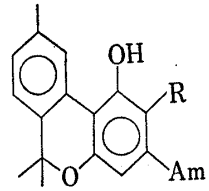
cannabidiolic acid (CBDA) R=COOH
cannabidiol (CBD) : R=H



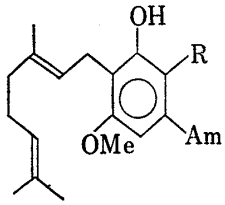
Δ^2-tetrahydrocannabinolic acid (THCA) : R=COOH
Δ^2-tetrahydrocannabinol (THC) : R=H



cannabichromenic acid (CBCA) : R=(COOH)
cannabichromene (CBC) : R=H



cannabinolic acid (CBNA) : R=COOH
cannabinol (CBN) : R=H



cannabigerolic acid monomethyl ether (CBGAM) : R=COOH
cannabigerol monomethyl ether (CBGM) : R=H

Chart 1

1) Part IV: Y. Shoyama, A. Yamaguchi, T. Sato, T. Yamauchi and I. Nishioka, *Yakugaku Zsshi*, **89**, 842 (1969).
2) Location: *Katakasu, Fukuoka.*
3) Z. Krejči and F. Šantavý, *Acta Univ. Palackianae Olomuc.*, **6**, 59 (1955); *C.A.*, **50**, 12080 (1956).
4) O.E. Schultz and G. Haffner, *Arch. Pharm.*, **291**, 391 (1958); *Z. Naturforsch.*, **14b**, 98 (1959).
5) R. Mechoulam and Y. Gaoni, *Tetrahedron*, **21**, 1223 (1965).

acids however being unsuccessful. In the preceding communications,[6a,b] the authors described the isolation of Δ^2-tetrahydrocannabinolic acid (THCA)[7] with preparative thin-layer chromatography (TLC), and cannabichromenic acid (CBCA) through silica gel column. THCA was also isolated by Claussen, *et al.*[11] with the aid of counter current technique. This paper deals with the isolation of a new component, cannabigerolic acid monomethyl ether (CBGAM), together with other known acids, THCA, CBDA, and CBCA, by means of simple column chromatography, and is concerned with the study on CBNA which is found in the stored Cannabis.

Cannabigerolic Acid Monomethyl Ether (CBGAM)

According to the gas chromatogram of the benzene percolates of the fresh domestic and Mexican hemps (Fig. 1), "Minamioshihara No. 1" (M-1), one of the domestic strains, appeared to contain a new substance, whose retention time coincided with that of cannabigerol monomethyl ether (CBGM),[12] as a second major peak, next to that of Δ^2-tetrahydrocannabinol (THC), while, in the domestic "Kumamoto" and Mexican strains, this peak was detected very slightly, besides the major peak of THC. Since the phenols scarcely exist in the percolate of the fresh hemp,[6a] and the phenol carboxylic acids are decarboxylated to the phenols by heating in gas chromatograph unless a treatment such as trimethylsilation has been done beforehand,[6,9] the peak shown as CBGM seemed to suggest the existance of its parent acid, CBGAM. M-1, therefore, was used for the isolation of CBGAM.

Priorto silica gel chromatography (GLC), the percolate of M-1 was passed through polyamide column in order to remove chlorophyll. On the elution of silica gel column with hexane–ethyl acetate (5:1 v/v), the fractions of slight amount of THC, the mixture of CBGAM and THCA, homogeneous THCA, and of CBDA were obtained separately with the same solvent system, and finally that of CBCA with 3:1 v/v system. The mixture of CBGAM and THCA was again chromatographed on silver nitrate–silica gel column to give pure CBGAM, whose structure,

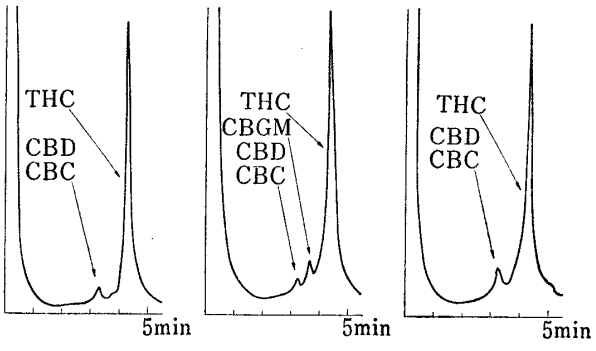


Fig. 1. Gas Chromatogram of Fresh Cannabis

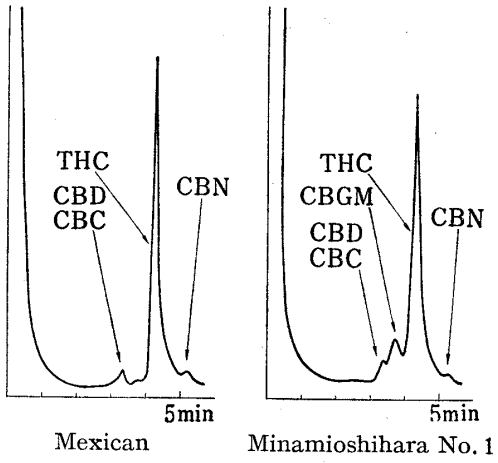


Fig. 2. Gas Chromatogram of Stored Cannabis

6) *a*) T. Yamauchi, Y. Shoyama, H. Aramaki, T. Azuma and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **15**, 1075 (1967); *b*) Y. Shoyama, T. Yamauchi and I. Nishioka, *ibid.*, **16**, 1157 (1968).
7) Δ^2-Tetrahydrocannabinolic acid (THCA) in this series corresponds with Δ^1-form by Mechoulam, *et al.*[8] and Δ^9-form by Claussen, *et al.*[9] Mechoulam, *et al.* reported the new type of THCA, *i.e.* THC acid B[10] which has not been detected in the strains cultivated in our University farm.
8) R. Mechoulam and Y. Shvo, *Tetrahedron*, **19**, 2073 (1963).
9) U. Claussen, W. Borger and F. Korte, *Ann.*, **693**, 158 (1966).
10) R. Mechoulam, Z. Ben-Zvi, B. Yagnitinsky and A. Shani, *Tetrahedron Letters*, **1969**, 2339.
11) U. Claussen, F.v. Spulak and F. Korte, *Tetrahedron* **24**, 1021 (1968).
12) T. Yamauchi, Y. Shoyama, Y. Matsuo and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1164 (1968).

on the location of carboxyl group, was assigned as in Chart 1 by the strongly deshielded chemical shift of phenolic proton in CBGAM methyl ester[5] (11.59 ppm). It was confirmed by the isolation of CBGAM from the fresh hemp that, as assumed precedingly,[12] not CBGM but its parent acid was a genuine substance in the plant.

Cannabinolic Acid

When the percolate of the stored Cannabis was examined by GLC (Fig. 2) and TLC CBNA was found together with THCA, CBDA, CBCA, and CBGAM, whereas in the fresh hemp the latter four substances were detected (Fig. 1). Although the isolation of pure CBNA from the plant source was unsuccessful owing to its low concentration, identification of this acid in the admixture with CBCA was carried out with the aid of nuclear magnetic resonance (NMR) as well as tlc and GLC. The presence of CBNA in the stored Cannabis suggested that CBNA is an artifact, and among the acids in the living plant, THCA seemed to be the most possible artificial precursor of CBNA according to their close structural relationship.

In order to study the possibility of the conversion of THCA to CBNA on the light, the former substance was irradiated with a UV lamp. The sample irradiated, afforded minor peak of CBNA on GLC, and the product was isolated with column chromatography, followed by the crystallization from hexane to give the fine needles of CBNA. It, on treatment with diazomethane, provided a methyl ester, physical constants of which were in good agreement with that by Mechoulam and Gaoni.[5] This conversion was also shown to take place under the irradiation in the ethanolic solution with the presence of chloranil. Contrary, Δ^3-THCA,[10,13] which was synthesized from CBDA with borontrifluoride etherate, was not converted to CBNA under the same condition as on THCA.

The effect of the temperature was then examined on the formation of CBNA. The samples of the powdered dried leaves were stored in the dark at room temperature for four months and at 35° for two months respectively. The analysis of the components indicated that at room temperature CBNA increased to 4.7% of the total cannabinoids. Under the latter condition, the dehydrogenation was accompanied by the decarboxylation to afford cannabinol (CBN), and the dehydrogenation shown as a total yield of CBNA and CBN was almost twice as much comparing with the former condition. When pure THCA was stored at 0° for 260 hours as a paste form, neither the dehydrogenation nor the decarboxylation to yield CBNA, THC, or CBN was observed. At 10—15°, however, *ca.* 7% of the original THCA was dehydrogenated to provide both CBNA and CBN.

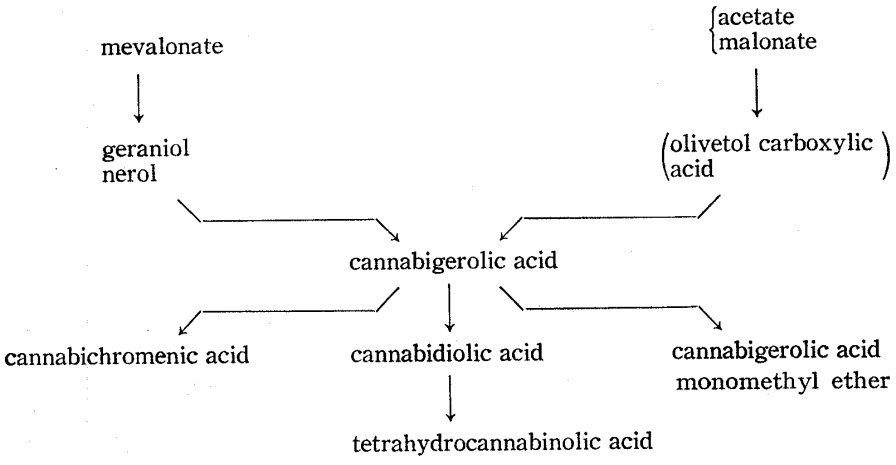


Chart 2. Biogenesis of Cannabinoids

13) The presence of this compound in nature is expected since Δ^3-THC was isolated in the hashish in U.S.A.[14]
14) R.L. Hively, W.A. Mosher and F.W. Hoffmann, *J. Am. Chem. Soc.*, **88**, 1832 (1966).

It is quite obvious therefore that the formation of CBNA from THCA progresses artificially by the light and by the higher temperature, CBNA being produced during the storage. With regard to the biogenesis of cannabinoids, Mechoulam and Gaoni[5] presented the pathway from CBGA (and cannabigerol) to CBNA (CBN) through CBDA (cannabidiol) and THCA (THC). As a result of our investigations, CBNA as well as the other phenols is to be excluded from the original cannabinoids in the living plants. CBGAM seems to arise from CBGA, and the possible scheme is given in Chart 2. The enzyme activity of CBCA formation appears quite remarkable during one or two weeks after seedling[6b] and then the formation of CBDA and THCA becomes predominant. The high concentration of CBGAM may be limited to some strains.

Experimental[15]

Column Chromatographic Isolation of the Phenol Carboxylic Acids in M-1——Fresh leaves of M-1 (1.0 kg) were dried in the shade within one week after harvest, powdered and percolated with benzene. The solvent (8 liters) was evaporated *in vacuo* under 40°, the residue was dissolved in ether, and a small amount of polyamide was added. The mixture was dried *in vacuo*, placed on top of a polyamide column (5 × 30 cm), and eluted with MeOH–water. The fractions containing the cannabinoids were combined, dissolved in acetone, and chilled at 0°. The deposit was removed by decantation and acetone was evaporated *in vacuo*. The

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

	Rf value		Color with[a] diazotizing benzidine	Relative retention[b] time
	Solv. 1	Solv. 2		
CBD	—	0.60	orange	1.00[c] (1.00)[d]
CBDA	0.67	—	orange	1.00 (2.38)
THC	—	0.57	orange red	1.23
THCA	0.59	—	red	1.23 (3.72)
Δ^3-THC	—	0.60	orange red	1.15
Δ^3-THCA	0.63	—	red	1.15 (3.56)
CBG	—	0.37	orange yellow	1.51
CBGA	0.67	—	orange yellow	1.51
CBGM	—	0.82	orange	1.08
CBGAM	0.59	—	red	1.08 (4.56)
CBC	—	0.44	brownish red	1.00
CBCA	0.24	—	brownish red	1.00 (3.84)
CBN	—	0.53	redaish violet	1.51
CBNA	0.20	—	redaish violet	1.51 (4.71)
CBP[e]	—	0.60	orange red	0.81
CBPA	0.65	—	red	0.81

a) J.E. Koch and W. Krieg, *Chem. Zentr.*, **62**, 140 (1938), *cf.* T. Furuya, *Kagaku No Ryoiki*, **59**, 91 (1964).
b) Figures in parenthesis indicate R.t_R values of the trimethyl silates.
c) retention time: 3.3 min
d) retention time: 2.0 min
e) cannabipinol (=cannabicyclol)

15) Melting points were taken on a Kofler block and are uncorrected. Infrared (IR) spectra were obtained with DS-301 spectrophotometer (Japan Spectroscopic Co. Ltd.). NMR spectra were recorded at 60 Mcps with a JNM-C-60H spectrometer (JEOL) in $CDCl_3$ solution, tetramethylsilane being used as internal standard, and mass spectra were measured with a JMS-01SG spectrometer (JEOL), GLC was conducted according to the method previously described.[6] On the quantitative analysis, cholestane was used as an internal standard. Thin-layer plate was prepared with Kiesel Gel G (Merck), and developed with hexane–EtOAc (1:1 v/v) for phenol carboxylic acids (Solv. 1), benzene for phenols (Solv. 2), and with hexane–benzene–diethylamine (25:10:1 v/v)[16] for phenols (Solv. 3). In column chromatography "Kanto" silica gel (100—200 mesh) was employed.

16) H. Aramaki, N. Tomiyasu and H. Yoshimura, *Chem. Pharm. Bull.* (Tokyo), **16**, 822 (1968).

residue (4.2 g) was dissolved in hexane and chromatographed on silica gel column (silica gel 420 g) using hexane–EtOAc (5:1—3:1 v/v). Fr. 1 (5:1 v/v hexane–EtOAc, 100 ml): phenol, trace *Rf*: 0.57 (Solv. 2), R.t_R[17] 1.23 (Table I); Fr. 2 (5:1, 100 ml): 700 mg, *Rf*: 0.59 (Solv. 1), R.t_R 1.08, 1.23; Fr. 3 (5:1, 300 ml): 1.80 g, *Rf*: 0.59 (Solv. 1), R.t_R 1.23; Fr. 4 (5:1, 300 ml): 30 mg, *Rf*: 0.67 (Solv. 1), R.t_R 1.00; Fr. 5 (3:1, 500 ml): 80 mg, *Rf*: 0.24 (Solv. 1), R.t_R 1.00.

CBGAM——Fr. 2 was rechromatographed on the column of 10% silver nitrate in silica gel (350 g) using hexane–EtOAc (5:1 v/v). The first fraction (Fr. 1′) eluted with 300 ml of the solvent gave 20 mg of homogeneous colorless paste, which indicated a single spot and peak on tlc (*Rf*: 0.59, Solv. 1) and on glc (R.t_R 1.08), respectively. UV λ_{max}^{EtOH} mμ (ε): 221 (17360), 260 (4770), 300 (2110). On boiling with toluene for 7 hr, Fr. 1′ was decarboxylated to give a phenol which was identified with the authentic sample of CBGM.[12]

Fr. 1′, on treatment with diazomethane in ether with usual method, provided a methyl ester. Mass Spectrum Calcd. for $C_{24}H_{36}O_4$ (CBGAM methyl ester): 388.261. Found: 388.256. UV λ_{max}^{EtOH} mμ (ε): 223 (18000), 267 (10000), 306 (3800). NMR δ: 0.90 (3H, ω-CH_3), 1.55, 1.64, 1.77 (3H each, $C_{8,9,10}$–CH_3), 3.32 (1H, C_1–H), 3.38 (3H, $C_{6'}$–OCH_3), 3.90 (3H, $C_{3'}$–$COOCH_3$), 4.95, 5.30 (2H, $C_{2,6}$–H), 6.27 (1H, $C_{5'}$–H), 11.59 (1H, $C_{2'}$–OH).

On successive elution of the $AgNO_3$–silica gel column with the 5:1 system, 180 mg of the mixture of THCA and CBGAM (Fr. 2′) and 300 mg of THCA (Fr. 3′) were obtained.

Other Acids——THCA, CBDA, and CBCA were obtained from Fr. 3, Fr. 4, and Fr. 5 respectively. These compounds were identified with the authentic samples[6] according to TLC, GLC, and other physical and chemical properties.

Cannabinolic Acid in the Stored Cannabis——The dried leaves of Mexican strain (1.0 kg), stored for 4 months, were worked up in the same manner described above. On silica gel column, 1.5 g of the acetone-treated residue (total 4.0 g) was chromatographed: Fr. 1 (5:1 v/v hexane–EtOAc, 400 ml): trace, *Rf*: 0.57 (Solv. 2), R.t_R 1.23; Fr. 2 (5:1, 300 ml): 520 mg, *Rf*: 0.59 (Solv. 1), R.t_R 1.23; Fr. 3 (3:1, 300 ml): trace, *Rf*: 0.67 (Solv. 1), R.t_R 1.00; Fr. 4 (2:1, 400 ml): 60 mg, *Rf*: 0.24 (Solv. 1), R.t_R 1.00; Fr. 5 (1:1, 300 ml): 20 mg, *Rf*: 0.24, 0.20 (Solv. 1), R.t_R 1.00, 1.51.

On boiling Fr. 5 with toluene for 7 hr, cannabichromene (CBC) (*Rf*: 0.44, Solv. 2) and CBN (*Rf*: 0.53, Solv. 2) were detected on TLC. Fr. 5 was methylated with diazomethane and the resulting methyl ester was submitted to NMR analysis. NMR δ: 0.95, 1.42, 1.64, 2.44 (C_{10}–CH_3 of CBNA), 3.95, 4.00 (–$COOCH_3$ of CBNA and CBCA), 5.51 (doublet, J=10.5 cps, C_2–H of CBCA), 6.26 ($C_{5'}$–H of CBCA), 6.43 ($C_{5'}$–H of CBNA), 6.80 (doublet, J=10.5 cps, C_1–H of CBCA), 7.17 ($C_{4,5}$–H of CBNA), 8.50 (C_2–H of CBNA).

Irradiation of THCA——a) Isolation of CBNA: The solution of 2.3 g of THCA in ether was poured into petre dish, and the solvent was evaporated. THCA in the dish was irradiated by 200W high pressure mercury lamp, in the distance of 40 cm from the source, at room temperature, and checked by the time with glc and TLC. After 260 hr irradiation, the paste was subjected to column chromatography with usual manner. The first fraction eluted with 5:1 hexane–EtOAc gave 920 mg of recovered THCA. On successive elution with 1:1 hexane–EtOAc, 190 mg of homogeneous paste was obtained, and on the crystallization from hexane it gave the prisms, mp 158—160° (decomp.). *Anal.* Calcd. for $C_{22}H_{26}O_4$ (cannabinolic acid): C, 74.55; H, 7.39. Found: C, 74.34; H, 7.40. R.t_R of trimethylsilate 4.71, UV λ_{max}^{EtOH} mμ (ε): 258 (14000), 294 (4900), 328 (2500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3470, 2700—2500 (sh), 870.

CBNA was esterified in the usual manner and the methyl ester was crystallized from hexane to give the prisms; mp 82—85.5° (decomp.), (mp 86—87° by Mechoulam and Gaoni).[5] *Anal.* Calcd. for $C_{23}H_{28}O_4$ (cannabinolic acid methyl ester): C, 74.97; H, 7.66. Found: C, 75.11; H, 7.80. UV λ_{max}^{EtOH} mμ (ε): 220 (28680), 267 (52400), 300 (13100), 330 (7300). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3620, 1720, 1641, 1610, 873. NMR δ: 0.91 (3H, ω-CH_3), 1.60 (6H, $C_{8,9}$–CH_3), 2.38 (3H, C_{10}–CH_3), 3.85 (3H, –$COOCH_3$), 6.38 (1H, $C_{5'}$–H), 7.08 (2H, $C_{4,5}$–H), 8.45 (1H, C_2–H), 12.77 (1H, $C_{2'}$–OH).

b) Irradiation of THCA in Ethanol: To the solution of 10 mg of THCA in 3 ml of ethanol, 10 mg of chloranil was added. The solution was placed in the quarz cell, irradiated at room temperature for 5—20 hr with the ultraviolet spectrum (UV) light in the distance of 18.5 cm from the source and the reactant was examined by TLC and quantitatively by GLC. The ratios of CBNA to THCA were 9/91 at 5 hr, 15/85 at 10 hr and 20/80 at 20 hr, and no phenols were found by TLC.

Δ^3-THCA——To the solution of 210 mg of CBDA in 10 ml of dioxane, 1 ml of BF_3 etherate was added. The solution was allowed to stand at room temperature for 7.5 hr, diluted with water and the mixture was extracted with ether. The ether layer was washed with water, dried over Na_2SO_4 and the solvent was evaporated *in vacuo* to give 200 mg of Δ^3-THCA as a paste, whose purity was proved by GLC of the trimethylsilate and tlc of the methyl ester, and also determined, after decarboxylation by heating with toluene, on tlc with $AgNO_3$-silica gel plate.[14] R.t_R of trimethylsilate : 3.56, UV λ_{max}^{MeOH} mμ (ε): 224 (15500), 270 (7030), 302 (3230). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3590, 1720, 1640, 1615. $[\alpha]_D^{23}$ −202° (c=0.9, $CHCl_3$). Δ^3-THCA (110 mg) was treated with diazomethane in the usual way, and the reactant was purified with silica gel column chromatography to give Δ^3-THCA methyl ester (37 mg). *Rf*: 0.67 (Solvent: hexane–$CHCl_3$–EtOAc 40:2:1 v/v,

17) Relative retention time.

THCA methyl ester 0.56). Mass Spectrum *m/e*:372 (M^+), UV λ_{max}^{MeOH} mμ (ε): 223 (32300), 272 (18700), 307 (7760). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1730, 1645, 1620. $[\alpha]_D^{23}$ $-277°$ (c=2.1, $CHCl_3$). NMR δ: 0.90 (3H, ω-CH_3), 1.02, 1.40 (3H each, $C_{8,9}$-CH_3), 1.73 (3H, C_{10}-CH_3), 3.93(3H, -$COOCH_3$), 5.40 (1H, C_4-H), 6.23 (1H, $C_{5'}$-H), 12.23 (1H, $C_{2'}$-OH).

The effect of the light on Δ^3-THCA was examined in EtOH in the presence of chloranil under the same condition as on THCA. After 20 hr, neither CBNA nor phenols were observed on glc and TLC.

The Effect of the Temperature——a) On the Leaves: Dried leaves (Mexican) were powdered and stored at room temperature (10—15°) for 4 months (Condition 1) and at 35° for 2 months (Condition 2), respectively. The sample was percolated with benzene and the percolate was extracted with 5% NaOH. The alkaline water layer was acidified and extracted with benzene (phenol carboxylic acid fraction). The benzene percolate, after extraction with 5% NaOH, was washed with water, dried over Na_2SO_4 and benzene was evaporated *in vacuo* (phenol fraction). The both fractions were quantitatively analysed by GLC. Condition 1: CBDA and CBCA: 5.6%, THCA: 82.6%, CBNA: 4.7%, CBD and CBC: 0.6%, THC: 5.9%, CBN: 0.6%. Condition 2: CBDA and CBCA: 4.5%, THCA: 20.1%, CBNA: 5.4%, CBD and CBC: 2.8%, THC: 60.9%, CBN: 6.3%.

b) On THCA: The solution of THCA in ether was placed in quarz glass tube. After the solvent was evaporated and the residual THCA was dried *in vacuo*, the tube was sealed and stored for 260 hr at 0° in the dark, and at room temperature in the dark and under UV light in the distance of 40 cm from the source, respectively. The formation of CBN and CBNA were examined by glc and tlc. The ratios of CBNA (CBN) to THCA (THC) were 0/100 (THCA only) at 0° in the dark, 7 (CBNA+, CBN+)/93 (THCA卄, THC±) at room temperature in the dark, and 47(CBNA卄, CBN±)/53(THCA only) at room temperature under UV light.

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