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Cannabis. VI.[1] Cannabicyclolic Acid[2]

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A new cannabinoid named cannabicyclolic acid (I) was isolated from the stored Cannabis, harvested early in the vegetative phase, and synthesized from cannabichromenic acid by means of ultraviolet light irradiation.

Cannabicyclolic acid is not an original cannabinoid but an artificial product, converted from cannabichromenic acid by natural irradiation during the storage of the Cannabis.

In the preceding paper of this series,[1] the authors reported the isolation of cannabinolic acid (CBNA) in the stored Cannabis and its artificial production from tetrahydrocannabinolic acid (THCA). Our recent investigation has proved the presence of a new phenol carboxylic acid, cannabicyclolic acid (CBLA), in the same source. This paper deals with the isolation and structural elucidation of this new component and its photochemical conversion from cannabichromenic acid (CBCA).[4]

When the methyl esters of the mixture of the phenol carboxylic acids obtained from the stored Cannabis were examined by thin-layer chromatograph (TLC), a new red spot (*Rf* 0.57), together with other known compounds, was observed with diazotized benzidine reagent,[5] being not detected in those from the fresh samples (Fig. 1).

The mixture of phenol carboxylic acids was chromatographed on silica gel in the same manner described precedingly,[1] and the optically inactive substance, $C_{22}H_{30}O_4$, mp 152—155°, was afforded as colorless prisms (A). A showed absorption of carboxylic group at 1665 cm^{-1} in the infrared spectrum (IR). In the nuclear magnetic resonance spectrum (NMR) of the methyl ester of A, the signal of a methoxycarbonyl group at 3.93 ppm and of a phenolic proton at 11.83 ppm indicated the chelation between each group. Therefore, the methoxycarbonyl group was assigned at *ortho* position of phenolic group of olivetol moeity.

On gas-liquid chromatography (GLC) (A) gave a single peak whose retention time coincided with that of cannabicyclol (CBL), in analogy with the cases of other phenol carboxylic acids of cannabinoids.[6,7]

On decarboxylation of A with heating at 150—160° followed by recrystallization, colorless prisms (B) were obtained. The melting point, ultraviolet (UV) and IR spectra of B were identical with those of CBL. Thus, A appeared to be a parent acid of CBL, and was named cannabicyclolic acid (CBLA).

CBL was isolated from the Cannabis as a minor component by Claussen, *et al.*[8] and by Mechoulam, *et al.*[9] respectively and the structure was first proposed by them as III

1) Part V: Y. Shoyama, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **18**, 1327 (1970).
2) This work was presented at the 90th Annual Meeting of Pharmaceutical Society of Japan, Sapporo, July 1970, "Abstracts of Papers," II, p. 213.
3) Location: *Katakasu, Fukuoka*; *a*) Present address: *Nanakuma, Fukuoka* (*Faculty of Pharmaceutical Sciences, Fukuoka University*).
4) Y. Shoyama, T. Fujita, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **16**, 1157 (1968).
5) J.E. Koch and W. Kreg, *Chem. Zentr.*, **62**, 140 (1938); *cf.* T. Furuya, *Kagaku No Ryoiki*, **59**, 91 (1964).
6) T.W.M. Davis, C.G. Farmilo, and M. Osadchuk, *Anal. Chem.*, **35**, 751 (1963).
7) Y. Shoyama, S. Yamaguchi, T. Sato, T. Yamauchi, and I. Nishioka, *Yakugaku Zasshi*, **89**, 842 (1969).
8) U. Claussen, F. von Spulak, and F. Korte, *Tetrahedron*, **24**, 1021 (1968).
9) R. Mechoulam and Y. Gaoni, Fortschr, *Chem. Org. Naturstoffe*, **25**, 175 (1967).

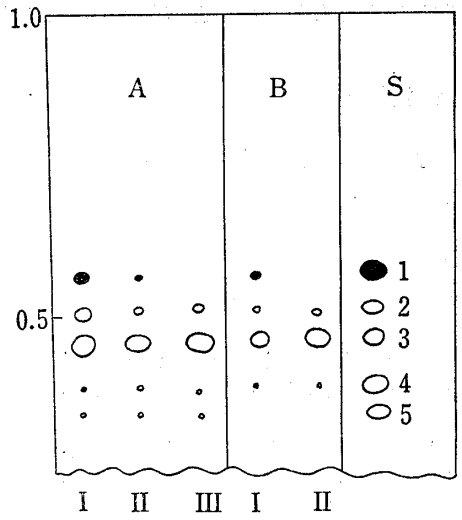

Fig. 1. Thin-Layer Chromatograms (on Silica gel G) Hexane–$CHCl_3$–EtOAc (40: 2: 1)

S: specimen samples
1: methyl cannabicyclolate
2: methyl cannabichromenate
3: methyl tetrahydrocannabinolate
4: methyl cannabidiolate
5: methyl cannabigerolate
A: Kumamoto strain
I: stored for 4 months (v)
II: stored for 7 months (r)
III: fresh leaves (r)
B: Mexican strain
I: stored for 4 years (r)
II: fresh leaves (r)
(r): harvested in the reproductive period
(v): harvested in the vegetative phase

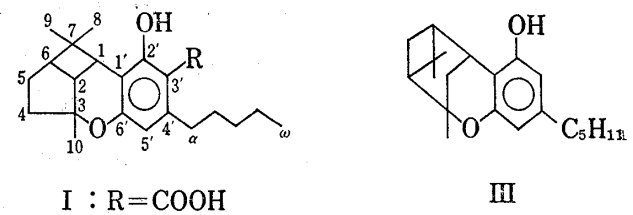

Chart 1

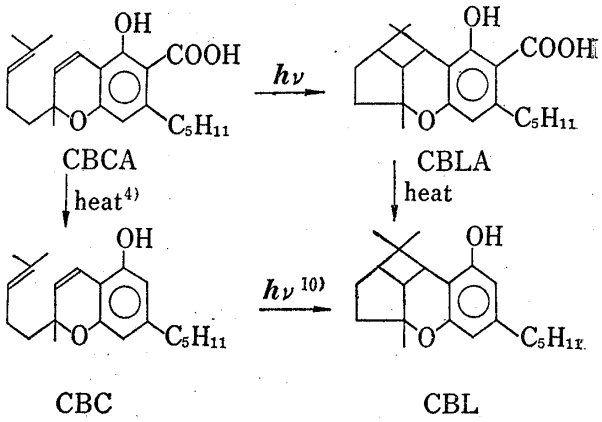

Chart 2

mainly on the basis of the mass spectrum data and synthetic study, then the structure (II) was presented according to the examination of NMR and of photochemical conversion of cannabichromene (CBC) to CBL,[10] and finally confirmed *via* X-ray study of dibromocannabicyclol.[11] The structure of A, therefore, was decided as CBLA (I).

CBLA was observed to exist in larger amount when Cannabis was harvested early in the vegetative phase[4] and stored, comparing with when havrested in the reproductive phase. The amount of CBLA indeed increased with storing of Cannabis. These facts, as well as the conversion of CBC to CBL,[9 10] suggested that CBCA seemed to be the most possible original substance of CBLA.

In order to elucidate the possible conversion of CBCA to CBLA on light exposure, CBCA was dissolved in dioxane–acetone and irradiated with a high pressure mercury arc lamp. The irradiation mixture was purified on silica gel column chromatography and the product afforded the peak of CBL on GLC and was crystallized from hexane–chloroform to give the fine colorless prisms, $C_{22}H_{30}O_4$, mp 154—156°, physical constants of which, were all in good agreement with those of CBLA isolated from the stored Cannabis. Furthermore the methylester, $C_{23}H_{32}O_4$, mp 89—90°, was also identified with CBLA-methylester by comparison of UV, IR, NMR spectrum and mixed melting point.

It is obvious that CBLA is not a genuine substance but an artificial product, converted from CBCA by natural irradiation during the storage and hence CBLA, as well as cannabi-

10) L. Crombie, R. Pinsford, A. Shani, B. Yagnitinsky, and R. Mechoulam, *Tetrahedron Letters*, **1968**, 5771; Y. Gaoni and R. Mechoulam, *J. Am. Chem. Soc.*, **93**, 217 (1971).
11) M.J. Begley, D.G. Clarke, L. Crombie, and D.A. Whiting, *Chem. Commun.*, **1970**, 1547.

nolic acid,[1] cannabielsoic acid A[12] and the neutral cannabinoids (phenols),[13] is to be excluded from the original cannabinoids in the living plant.

Experimental[14]

TLC Examination of the Phenol Carboxylic Acid (PCA) Fraction from the Cannabis——Each lot of the Cannabis was percolated with benzene and the solvent was evaporated *in vacuo*. To the residue 5 ml of ethereal diazomethane was added and allowed to stand for 30 min. After evaporation of ether the residue was examined on TLC (Fig. 1).

Isolation of CBLA (I) from the Stored Cannabis——The dried leaves of Kumamoto strain (2.2 kg) stored for 4 months were powdered and percolated with benzene. The benzene extract was treated with acetone at 0° and the insoluble fraction was removed by filtration. Acetone was evaporated *in vacuo* and the residue (172 g) was chromatographed on a polyamide (850 g) column using MeOH–water (1:2—6:1 v/v) as solvent. The fractions containing the phenol carboxylic acids were combined and the solvent was evaporated *in vacuo*. The residue (21 g) was rechromatographed on a silica gel (210 g) column with hexane–EtOAc (5:1—3:1 v/v) as solvent and following fractions were obtained. Fr. 1 (5:1): neutral cannabinoids (trace); Fr. 2 (5:1): (I) and THCA (470 mg); Fr. 3 (5:1): THCA (7.0 g); Fr. 4 (3:1): THCA, CBDA and CBCA (450 mg); Fr. 5 (3:1): CBCA and CBGA (2.0 g). Fr. 2 (470 mg) was rechromatographed on a silica gel (47 g) column using hexane–EtOAc (5:1—3:1 v/v) as solvent and the fraction eluted with hexane–EtOAc (5:1) gave 60 mg of colorless paste indicating homogeneity by TLC and GLC. It was crystallized from hexane–chloroform to give the prisms, mp 152—155° (decomp.), $[\alpha]_D^{16}=0°$ ($c=0.33$, chloroform). *Anal.* Calcd. for $C_{22}H_{30}O_4$ (CBLA): C, 73.71; H, 8.44. Found: C, 73.62; H, 8.60. UV λ_{max}^{MeOH} mμ (ε); 227.5 (24000), 271 (9700), 306 (3500). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3080—3260 (OH), 1665 (C=O).

CBLA-Methyl Ester——On treatment with diazomethane in ether with usual method (I) provided a methyl ester, prisms (MeOH), mp 87—90°. *Anal.* Calcd. for $C_{23}H_{32}O_4$ (CBLA-Me): C, 74.16; H, 8.66. Found: C, 74.49; H, 8.97. Mass Spectrum Calcd. for $C_{23}H_{32}O_4$: 372.230. Found: 372.227. UV λ_{max}^{MeOH} mμ (ε): 228 (20740), 275 (11850), 308 (4070). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200 (OH), 1730 (C=O). NMR (in $CDCl_3$, 100 MHz) ppm: 0.78 (3H, C_8 or C_9-CH_3), 0.90 (3H, ω -CH_3), 1.40 (6H, C_{10} and C_8 or C_9-CH_3), 2.30—2.50 (1H, C_6-H), 2.58 (1H, quartet, $J=9.5$ cps and 7.0 cps, C_2-H), 2.73—2.93 (2H, α-CH_2), 3.16 (1H, doublet, 9.5 cps, C_1-H), 3.93 (3H, -$COOCH_3$), 6.31 (1H, C'_5-H), 11.83 (1H, C'_2-OH).

Decarboxylation of CBLA——(a) (I) (20 mg) was heated at 150—160° for 15 min and the product was crystallized from hexane–chloroform to give the prisms, mp 146—147°. $[\alpha]_D^{25}$ 0° ($c=0.57$, chloroform). Mass Spectrum *m/e* 314 (M$^+$). UV λ_{max}^{MeOH} mμ (ε): 276 (1800), 283.5 (1800). IR ν_{max}^{KBr} cm^{-1}: 3375 (OH). On admixture with CBL isolated from the stored Cannabis, no depression of melting point was observed. (b) On boiling with toluene for 8 hr (I) was decarboxylated to give a neutral cannabinoid which was identified with CBL.

Irradiation of CBCA——The solution of CBCA (300 mg) in 70 ml of dioxane and 10 ml of acetone was placed in a Pyrex vessel and irradiated with a high pressure mercury arc lamp (UVL-700P, 100V, 100 W) under a current of nitrogen at 30° for 25 hr. The reaction mixture was chromatographed on a silica gel (30 g) column using hexane–EtOAc (5:1—3:1 v/v) as solvent and the following fraction was obtained. Fr. 1 (5:1) 58 mg: Fr. 2 (3:1) 120 mg (CBCA). Fraction 1 (58 mg) was crystallized from hexane–chloroform to give the prisms, mp 154—156°, $[\alpha]_D^{16}$ 0° ($c=0.25$, chloroform). *Anal.* Calcd. for $C_{22}H_{30}O_4$ (CBLA): C, 73.71; H, 8.44. Found: C, 73.45; H, 8.48. This specimen was identified with authentic CBLA, by mixed mp, TLC, UV and IR spectra.

Fraction 1 was treated with diazomethane–ether in the usual manner and the product was crystallized from methanol to give prisms, mp 89—90°. Mass spectrum Calcd. for $C_{23}H_{32}O_4$: 372.230. Found: 372.229. UV λ_{max}^{MeOH} mμ (ε): 228.5 (20240), 275 (10240), 309 (3660). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3200 (OH), 1725 (C=O). NMR (in $CDCl_3$, 60 MHz) ppm: 0.78 (3H, C_8 or C_9-CH_3), 0.91 (3H, ω-CH_3), 1.40 (6H, C_{10} and C_8 or C_9-CH_3), 3.91 (3H, -$COOCH_3$), 6.26 (1H, C'_5-H), 11.73 (1H, C'_2-OH). On admixture with CBLA-methylester no depression of melting point was observed.

12) A. Shani and R. Mechoulam, *Chem. Commun.*, **1970**, 273.

13) Cannabigerol, cannabidiol, cannabinol, tetrahydrocannabinol, cannabichromene, cannabigerol monomethyl ether, *etc.*

14) Melting points were taken on a Kofler block and are uncorrected. IR spectra were obtained with a Koken DS-301 and UV spectra were recorded with a Shimazu SV-50A. NMR spectra were recorded at 100 MHz with a JNR-4H-100 and at 60 MHz with a JNM-C-60H (JEOL) in $CDCl_3$ solution, tetramethylsilane being used as internal standard, and Mass spectra were measured with a JMS-01SG spectrometer (JEOL). Thin-layer and gas-liquid chromatographies were conducted in the same way described in the preceding paper.[1]

Fraction 1 was decarboxylated in the same manner to give the prisms, mp 146—147°. $[\alpha]_D^{25}$ 0° (c= 0.94, chloroform). Mass Spectrum m/e 314: (M^+). UV λ_{max}^{MeOH} mμ (ε): 276 (1200), 283.5 (1200). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH). On admixture with CBL no depression of melting point was observed.

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