[Chem. Pharm. Bull. 26(12)3641—3646(1978)]

UDC 547.914.02.05 : 581.192

Cannabis. XIII.[1] Two New Spiro-compounds, Cannabispirol and Acetyl Cannabispirol

Yukihiro Shoyama and Itsuo Nishioka

Faculty of Pharmaceutical Sciences, Kyushu University[2]

(Received April 19, 1978)

Two new spiro-compounds, cannabispirol and acetyl cannabispirol, were isolated along with cannabispirone and cannabispirenone from the Japanese domestic cannabis and these structures were elucidated. The biogenetic relationship of spiro-compounds and cannabinoids was also discussed.

Keywords——moraceae; cannabis; spiro-compound; structure; biogenesis

Recently, various kinds of compounds besides cannabinoids have been isolated from cannabis by many investigators, like alkaloid,[3] triterpenoid,[4] essential oil[5] and spiro-compound, cannabispirone (II)[6] and cannabispirenone (III).[6]

This paper will document that the isolation and the structure elucidation of two new spiro-compounds and further present the biogenetic correlation of these compounds and cannabinoid acids.

As a continuative study on cannabis constituents, we have examined the neutral cannabinoid containing fraction (Fr. 1) which can be separated from cannabinoid acid fraction (Fr. 2) by polyamide column chromatography as previously done.[7] Since some phenolics which were more polar than the usual neutral cannabinoids were detected on thin-layer chromatography (TLC) of Fr. 1, these phenolic compounds were isolated by silica gel column chromatography and named as I, II, III and IV in order of decreasing polarity on TLC (Fig. 1).

Compound II and III were identified with cannabispirone (II)[6] and cannabispirenone (III),[6] respectively, by the comparisons with the physical constant reported.

Compound IV (cannabispirol), $C_{15}H_{20}O_3$, mp 194—197°, colorless needles, showed the positive coloration with diazotized benzidine (red). This ultraviolet (UV) spectrum indicated that IV had non-conjugated benzene ring at 276.5, 280 and 284 nm. The infrared (IR) spectrum of IV showed the absorption for two kinds of hydroxyl group at 3460 and 3280 cm^{-1} together with benzene ring at 1622 and 1601 cm^{-1}. The proton magnetic resonance (PMR) spectrum showed signals for one methoxyl group at 3.68 δ, an ethylene moiety at 2.16 δ (2H, t, J=7 Hz) and 2.96 δ (2H, t, J=7 Hz), one hydroxymethine proton at 4.35 δ (1H, t, J=4 Hz), one hydroxyl signal at 5.24 δ and a pair of doublet indicating *meta* coupling of aromatic protons at 6.54 δ (1H, d, J=3 Hz) and 6.70 δ (1H, d, J=3 Hz). This PMR spectrum was

1) Part XII: Y. Shoyama, H. Kushima, and I. Nishioka, Manuscript in preparation.
2) Location: *3-1-1 Maidashi, Higashiku, Fukuoka.*
3) H.L. Lotter, D.J. Abraham, C.E. Turner, J.E. Knapp, P.L. Schiff Jr., and D.J. Slatkin, *Tetrahedron Lett.*, **1975**, 2815.
4) D.J. Slatkin, N.J. Doorenbos, L.S. Harris, A.N. Masond, M.W. Quimby, and P.L. Shiff Jr., *J. Pharm. Sci.*, **60**, 1891 (1972).
5) *a*) L. Martin, D.M. Smith, and C.G. Farmilo, *Nature* (London), **191**, 774 (1961); *b*) M.C. Nigam, K.L. Handa, I.C. Nigam, and L. Levi, *Canad. J. Chem.*, **43**, 3372 (1965); *c*) E. Stahl and R. Kunde, *Tetrahedron Lett.*, **1973**, 2841.
6) *a*) T. Ottersen, A. Aasen, F.S. El-Feraly, and C.E. Turner, *Chem. Commun.*, **1976**, 580; *b*) C.A.L. Bercht, J.P.C.M. van Dongen, W. Heerma, R.J.J.Cl. Lousberg, and F.J.E.M. Juppers, *Tetrahedron*, **32**, 2939 (1976); *c*) F.S.El-Feraly, M.A. Elsohly, E.G. Boeren, C.E. Turner, T. Ottersen, and A. Aasen, *Tetrahedron*, **33**, 2732 (1977).
7) Y. Shoyama, R. Oku, T. Yamauchi, and I. Nishioka, *Chem. Pharm. Bull.* (Tokyo), **20**, 1927 (1972).

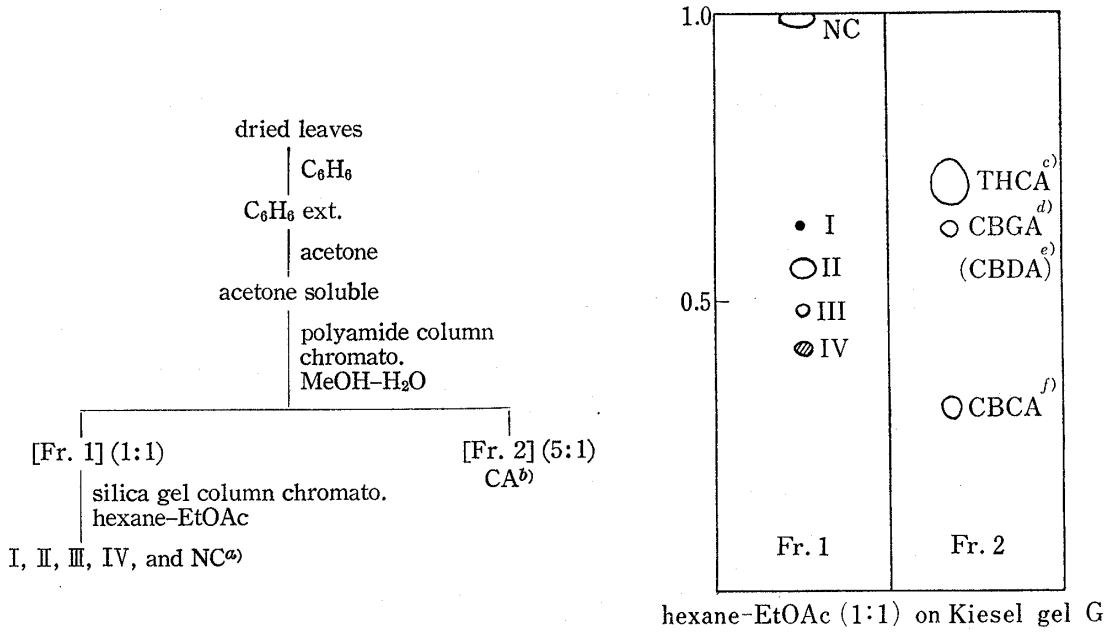

Fig. 1. Extraction, Purification and TLC of Spiro-compounds

a) neutral cannabinoids,
b) cannabinoids acids,
c) tetrahydrocannabinolic acid,
d) cannabigerolic acid,
e) cannabidiolic acid,
f) cannabichromenic acid.

similar to that of II except methylene region. This mass (MS) spectrum exhibited fragments, *m/e* 248 (M^+), 230, 202, 189 and 176. These spectral data suggested that IV may be an alcohol type of II having an axial hydroxyl group.

Upon acetylation, IV gave diacetate (IVa), colorless needles, $C_{19}H_{24}O_5$, mp 141—142°. Two types of acetoxyl absorption were observed by IR spectrum (1750 and 1722 cm^{-1}) and PMR spectrum (2.09 δ and 2.36 δ). Furthermore, the PMR spectrum demonstrates the existence of a secondary acetoxyl group in IVa by the signal at 5.08 δ (1H, t, J=4 Hz, acetoxymethine proton). The PMR decoupling experiments were carried out to confirm the existence of an ethylene moiety in IVa. Irradiation of benzyl methylene at 2.86 δ (2H, t, J=6 Hz) collasped the triplet at 2.00 δ to a singlet and *vice versa*.

On oxidation using Jones reagent, IV gave a colorless needles, $C_{15}H_{18}O_3$, mp 179—182°, which was identified with II by the direct comparison (UV, IR, PMR and MS). Therefore, it follows that IV corresponds to an alcohol type of II.

Concerning the stereostructure of IV, since the hydroxyl group of IV is axial, two possible structures (A and B) in 2 pairs of equilibria are proposed as shown in Fig. 2, that is, *syn* and *anti* between hydroxy group and indane skeleton.

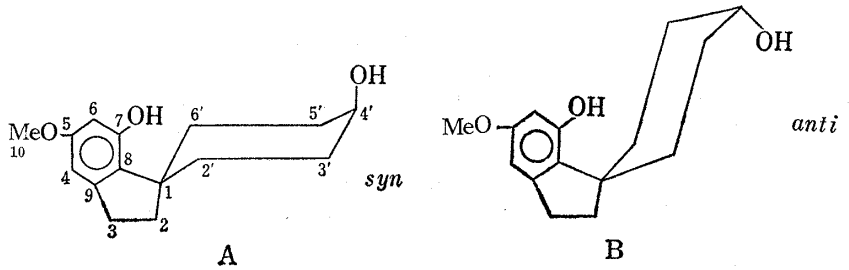

Fig. 2. Possible Structure of Cannabispirol (IV)

As pointed out by Roberts *et al.*,[8] the obvious difference was seen concerning chemical shift of each carbon on *cis* and *trans* 4-methyl cyclohexanol. In order to apply this rule for the determination of the stereostructure of IV, a pair of stereoisomer was obtained by the reduction of II using $NaBH_4$, that is, IV (minor product) and IV′ (major product), $C_{15}H_{20}O_3$, colorless needles, mp 178—180°. PMR spectrum of IV′ pointed out that the secondary hydroxyl group was obviously equatorial indicating a hydroxymethine proton at 4.06 δ (1H, m, $J=w$ h/2=20 Hz), compared with that of IV as previously shown.

The comparison of C-13 nuclear magnetic resonance (CMR) spectrum of IV and IV′ gives an evidence that the hydroxyl group of IV might be *syn* against indan skeleton in a full agreement with the reported results[8] as shown in Fig. 3. On the other hand, CMR data of IV′ explain that the hydroxyl group should be *anti* against indan skeleton.

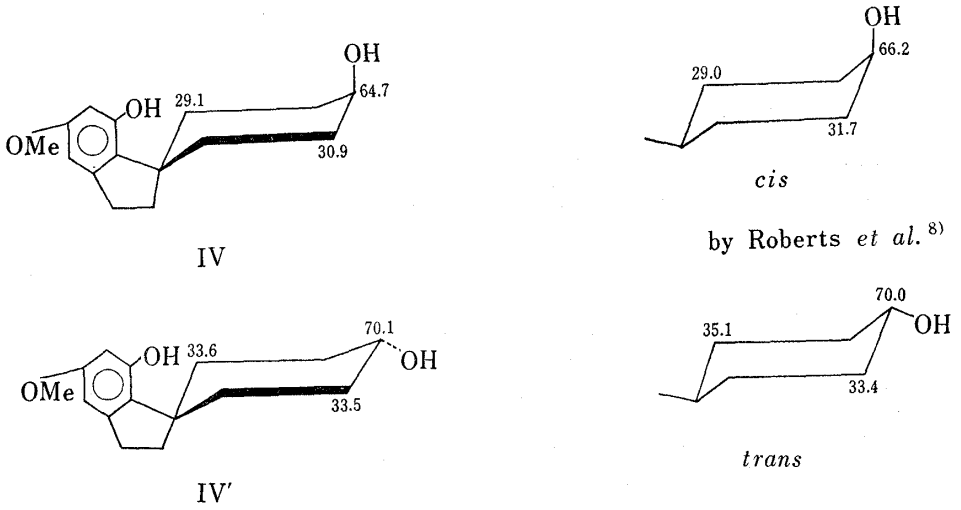

Fig. 3. Stereostructure of Cannabispirol (IV)

From above results, the stereostructure of IV has been determined as A in Fig. 2.

Compound I (acetyl cannabispirol), colorless needles, $C_{17}H_{22}O_4$, mp 184—185°, colored with diazotized benzidine (red). The UV and IR spectrum indicated that the structure of I might be similar to IV. The PMR spectrum exhibited one acetoxyl signal (2.10 δ), one acetoxymethine signal (5.04 δ, t, $J=4$ Hz).

Decoupling experiments provided additional evidence for this substitution pattern. Irradiation of the broad boublet around at 1.36 δ (2H, $J=14$ Hz, $C_{2'}$,$C_{6'}$ eq-H) changed the sextet centering at 2.44 δ (2H, $J=5$, 12, 14 Hz, $C_{2'}$,$_{6'}$ ax-H) into double doublet ($J=5$, 12 Hz). On the other hand, irradiation of the sextet at 2.44 δ collapsed the broad doublet at 1.36 δ to a triplet ($J=5$ Hz). Furthermore, the irradiation of the multiplet near 1.60—1.92 δ (4H, $C_{3'}$,$_{5'}$–H) changed the triplet at 5.40 δ (1H, t, $J=4$ Hz, $C_{4'}$–H) into a singlet (Fig. 4). These evidences strongly supported that I must be a mono acetylated IV indicating that the MS spectrum of I was similar to that of IV with the increase of 42 units.

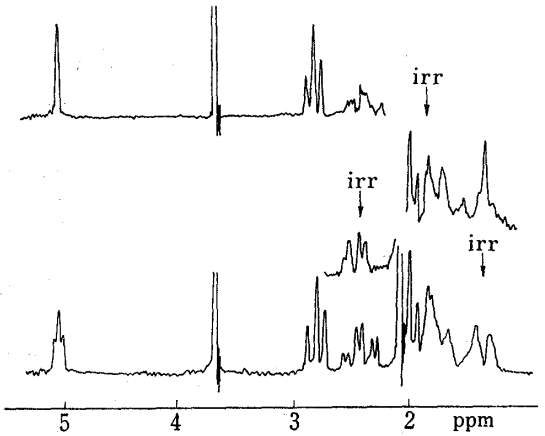

Fig. 4. PMR Spectrum of Acetyl Cannabispirol (I)

8) J.D. Roberts, F.J. Weigert, J.I. Kroschwitz, and H.J. Reich, *J. Am. Chem. Soc.*, **92**, 1338 (1970).

On partial acetylation using acetic acid and BF_3, IV afforded a monoacetate, colorless needles, mp 184—185°, which was identified with I by the direct comparison (UV, IR, PMR and MS).

Although previous communication[6] pointed out that cannabispirone and cannabispirenone were found in the specific strain like the Indian or the South African strain, we detected those spiro-compounds in all strain which were cultivated in this laboratory (the Minamioshihara No. 1 strain, the CBDA strain, other Japanese domestic strains, the Mexican strain and the Meao strain). However, the contents of those spiro-compounds were quantitatively different in individual cannabis plants.

Current interest in those spiro-compounds has been centered on the biogenetic relationship with cannabinoid acids[9] as summarized in Fig. 5. This studies will be investigated in future.

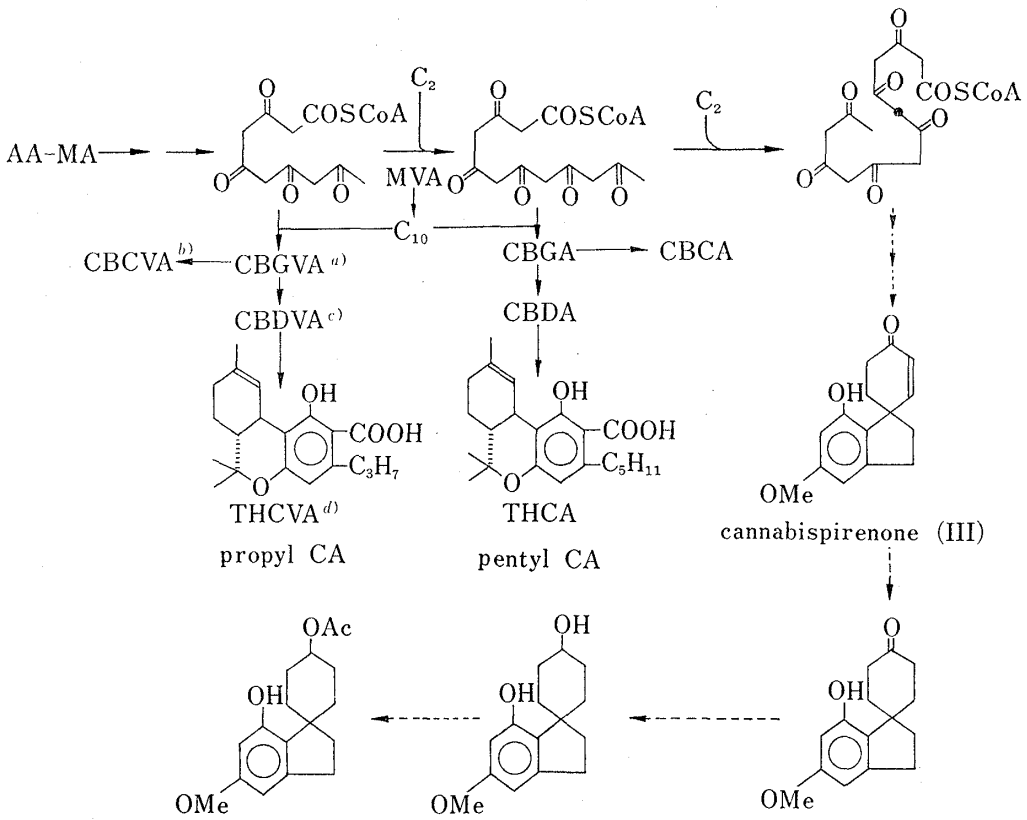

Fig. 5. Biogentic Correlation of Spiro-compounds and Cannabinoid Acids

a) cannabigerovarinic acid,
b) cannabichromevarinic acid,
c) cannabidivarinic acid,
d) tetrahydrocannbivarinic acid.

Experimental

Melting points were taken on a Kofler block and were uncorrected. UV spectra were determined by Hitachi 124 Spectrophotometer. IR spectra were obtained with a Nihon Bunko Model DS-301 Spectrometer. PMR spectra were taken in $CDCl_3$ or d_5-pyridine 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), m(multiplet), sex(sextet) and b(broad). CMR spectra were taken in $CDCl_3$ or d_5-pyridine solution at 25.05 MHz on a JNM-FX 100 Spectrometer.

9) Y. Shoyama, M. Yagi, I. Nishioka, and T. Yamauchi, *Phytochemistry*, **14**, 2189 (1975).

MS spectra were taken on a JEOL-JMS-01SG. Optical rotations were taken with a JASCO DIP-SL automatic polarimeter. TLC plate was prepared with Kieselgel G (Merck). Column chromatography was carried out with Kieselgel 60 (0.06—0.2 mm, Merck) using 50—100 times quantity of the material.

Extraction and Isolation (Fig. 1)——Air dried leaves (2.2 kg, Kumamoto strain) were extracted with benzene twice. The extractives were treated with cold acetone and then insoluble portion was removed by filtration. The filtrate was evaporated and then the residue was column chromatographed on a polyamide using 5 times quantity of the material eluting with MeOH–H_2O (1: 1) to give fraction 1 (containing neutral cannabinoids and spiro-compounds; 10 g) and then eluting with MeOH–H_2O (5: 1) to give fraction 2 (containing cannabinoid acids; 47.5 g). Fraction 1 was column chromatographed on a silica gel eluting with hexane–EtOAc (5: 1—1: 1) to give fraction 1-1 (containing I; 106 mg), fraction 1-2 (containing II; 419 mg), fraction 1-3 (containing III; 302 mg) and fraction 1-4 (containing IV; 244 mg).

Fraction 1-1 was recrystallized from benzene to give I, colorless needles, mp 184—185°, $[\alpha]_D^{20}$ +23.6 (c=1.21, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 273 (3.06), 277 (3.06), 281 (3.08). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1728, 1700 (C=O), 1617, 1598 (C=C). PMR (in $CDCl_3$) ppm: 1.36 (2H, bd, J=14 Hz, $C_{2',6'}$-eq H), 1.60—1.92 (4H, m, $C_{3',5'}$–H), 2.00 (2H, t, J=7 Hz, C_2–H), 2.10 (2H, s, Ac), 2.44 (2H, sex, J=5, 12, 14 Hz, $C_{2',6'}$-ax H), 2.81 (2H, t, J=7 Hz, C_3–H), 3.70 (3H, s, OMe), 5.04 (2H, t, J=4 Hz, $C_{4'}$–H, –OH), 6.11 (1H, d, J=3 Hz, arom. H), 6.30 (1H, d, J=3 Hz, arom. H). CMR ($CDCl_3$) ppm: 21.4 ($OCO\underline{C}H_3$), 27.1 (C-3′, 5′), 29.0 (C-2′,6′), 31.0 (C-3), 34.9 (C-2), 47.9 (C-1), 55.2 (C-10), 69.3 (C-4′), 100.7 (C-6), 101.7 (C-4), 134.0 (C-8), 146.1 (C-9), 153.1 (C-7), 159.7 (C-5), 170.9 ($O\underline{C}OCH_3$). MS m/e: 290 (M^+), 230, 215, 202, 189, 176, 161, MS Calcd. for $C_{17}H_{22}O_4$; 290.152. Found: 290.151.

Fraction 1-2 was recrystallized from $CHCl_3$–hexane to give II, colorless needles, mp 179—182°. II was identified by the comparison of the physical data of cannabispirone reported (UV, IR, PMR and MS). *Anal.* Calcd. for $C_{15}H_{18}O_3$: C, 73.14; H, 7.37. Found: C, 72.99; H, 7.35.

Fraction 1-3 was recrystallized from $CHCl_3$ to give III, mp 170—173°. III was identified by the comparison of the physical data of cannabispirenone reported (UV, IR, PMR and MS). *Anal.* Calcd. for $C_{15}H_{16}O_3$: C, 73.75; H, 6.60. Found: C, 73.61; H, 6.69.

Fraction 1-4 was recrystallized from EtOAc to give IV, colorless needles, mp 194—197°, $[\alpha]_D^{20}$ 0° (c=1.12, MeOH), UV λ_{max}^{MeOH} nm (log ε): 276.5 (2.93), 280 (2.93), 284 (2.95). IR ν_{max}^{KBr} cm^{-1}: 3460, 3280 (OH), 1622, 1601 (C=C), PMR (d_5-pyr.) ppm: 1.53 (2H, bd, J=12 Hz, $C_{2',6'}$-eq H), 1.80—2.10 (4H, m, $C_{3',5'}$–H), 2.16 (2H, t, J=7 Hz, C_2–H), 2.96 (2H, t, J=7 Hz, C_3–H), 3.48 (2H, sex, J=4, 12, 12 Hz, $C_{2',6'}$-ax H), 3.68 (3H, s, OMe), 4.35 (1H, t, J=4 Hz, $C_{4'}$–H), 5.24 (1H, bs, $C_{4'}$–OH), 6.54 (1H, d, J=3 Hz, arom. H), 6.70 (1H, d, J=3 Hz, arom. H), 11.15 (1H, bs, C_7–OH). CMR (d_5-pyr.) ppm: 29.1 (C-2′, 6′), 30.9 (C-3′, 5′), 31.3 (C-3), 35.2 (C-2), 49.1 (C-1), 55.0 (C-10), 64.7 (C-4′), 100.6 (C-6), 101.3 (C-4), 130.1 (C-8), 145.8 (C-9), 156.0 (C-7), 160.1 (C-5). MS m/e: 248 (M^+), 230, 215, 202, 189, 176. *Anal.* Calcd. for $C_{15}H_{20}O_3$: C, 72.35; H, 8.12. Found: C, 72.40; H, 8.21.

Acetylation of IV——IV (30 mg) was acetylated by Ac_2O–pyridine mixture to give crude IVa, which was purified by recrystallization from MeOH to give colorless needles, mp 141—142°. UV λ_{max}^{MeOH} nm (log ε): 273 (3.29), 280 (3.29). IR ν_{max}^{KBr} cm^{-1}: 1750, 1722 (C=O), 1612, 1585 (C=C). PMR (in $CDCl_3$) ppm: 1.38 (2H, bd, J=14 Hz, $C_{2',6'}$), 1.64—2.00 (4H, m, $C_{3',5'}$–H), 2.00 (2H, t, J=6 Hz, C_2–H), 2.09 (3H, s, OAc), 2.36 (3H, s, OAc), 2.86 (2H, t, J=6 Hz, C_3–H), 3.62 (3H, s, OMe), 5.08 (1H, t, J=4 Hz, $C_{4'}$–H), 6.42 (1H, d, J=3 Hz, arom. H), 6.64 (1H, d, J=3 Hz, arom. H). MS m/e: 332 (M^+), 290, 272, 230, 215, 202, 189, 187, 176 (base peak), 174, 163, 161 and 149. *Anal.* Calcd. for $C_{19}H_{24}O_5$: C, 68.56; H, 7.28. Found: C, 68.74; H, 7.38.

Jones Oxidation of IV——IV (252 mg) was dissolved in acetone (10 ml) and Jones reagent was added dropwise to the acetone solution until the changing color of the solution did not observed (approximately 0.2 ml). The reaction mixture was stirred for more 15 min at room temperature. The reactant was diluted with H_2O and extracted with EtOAc–hexane (1: 1). The organic layer was washed and then evaporated. The residue (205 mg) was recrystallized from hexane–benzene mixture to give colorless needles (II), mp 179—182°, which were identified by the direct comparison (UV, PMR and MS) with an authentic cannabispirone.

Reduction of II by $NaBH_4$——II (100 mg) was dissolved in MeOH (10 ml) and $NaBH_4$ (100 mg) was added. The mixture was allowed to stand for 1 hr at room temperature. The solvent was evaporated to dryness. The residue was suspended in H_2O and extracted with EtOAc 3 times. EtOAc extractives were repeatedly column chromatographed on silica gel eluting with benzene–acetone (5: 1) to give IV′ (54 mg) and IV (7.2 mg) which was identified with the authentic IV.

IV′, colorless needles, mp 177—180°. IR ν_{max}^{KBr} cm^{-1}: 3280, 3150 (OH), 1610, 1596 (C=C). PMR (d_5-pyr.) ppm: 1.60—2.00 (4H, m, $C_{3',5'}$–H), 2.12 (2H, t, J=7 Hz, C_2–H), 2.90 (2H, t, J=7 Hz, C_3–H), 3.66 (3H, s, OMe), 4.06 (1H, m, J=w h/2=20 Hz), 5.04 (1H, bs, $C_{4'}$–OH), 6.50 (1H, d, J=3 Hz, arom. H), 6.67 (1H, d, J=3 Hz, arom. H), 11.25 (1H, s, C_7–OH). CMR (d_5-pyr.) ppm: 31.2 (C-3), 33.5 (C-3′, 5′), 33.6 (C-2′, 6′), 35.8 (C-2), 48.3 (C-1), 55.2 (C-10), 70.1 (C-4′), 100.8 (C-6), 101.0 (C-4), 128.8 (C-8), 145.9 (C-9). MS m/e: 248 (M^+), 230, 215, 202, 189, 176, 161. MS Calcd. for $C_{15}H_{20}O_3$; 248.141. Found: 248.141.

Partial Acetylation of IV——IV (20 mg) was dissolved in AcOH (2.5 ml) and BF_3 ether solution (1 ml) was added. The mixture was stirred for 24 hr at room temperature. The reactant was diluted with H_2O and then repeatedly extracted with ether. The solvent was evaporated to give crude IVb which was passed

through silica gel column chromatography using benzene–acetone (10:1) as a solvent and then recrystallized from benzene–hexane to give pure IVb, colorless needles, mp 184—185°. IVb was identified by the direct comparison (UV, IR, PMR and MS) with an authentic I.

Acknowledgement The authors are grateful to Mr. T. Somehara and F. Yamato for their excellent technical assistance in a part of this study. Thanks are also due to Drs. H. Okabe, G. Nonaka, M. Yahara and K. Harano for the encouragement and useful discussion throughout this work and also due to Mr. N. Okamura, H. Matsui, Y. Tanaka, Miss M. Kawamura and Miss Y. Soeda and the members of the Central Analysis Room of this University for IR, UV, PMR, CMR, mass spectra measurements and microanalyses.