

Cannabis. XIII.¹⁾ Two New Spiro-compounds, Cannabisirol and Acetyl Cannabisirol

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Two new spiro-compounds, cannabisirol and acetyl cannabisirol, were isolated along with cannabispiron 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 oil⁵⁾ and spiro-compound, cannabispiron (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 cannabispiron (II)⁶⁾ and cannabispirenone (III),⁶⁾ respectively, by the comparisons with the physical constant reported.

Compound IV (cannabisirol), $C_{15}H_{20}O_3$, 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

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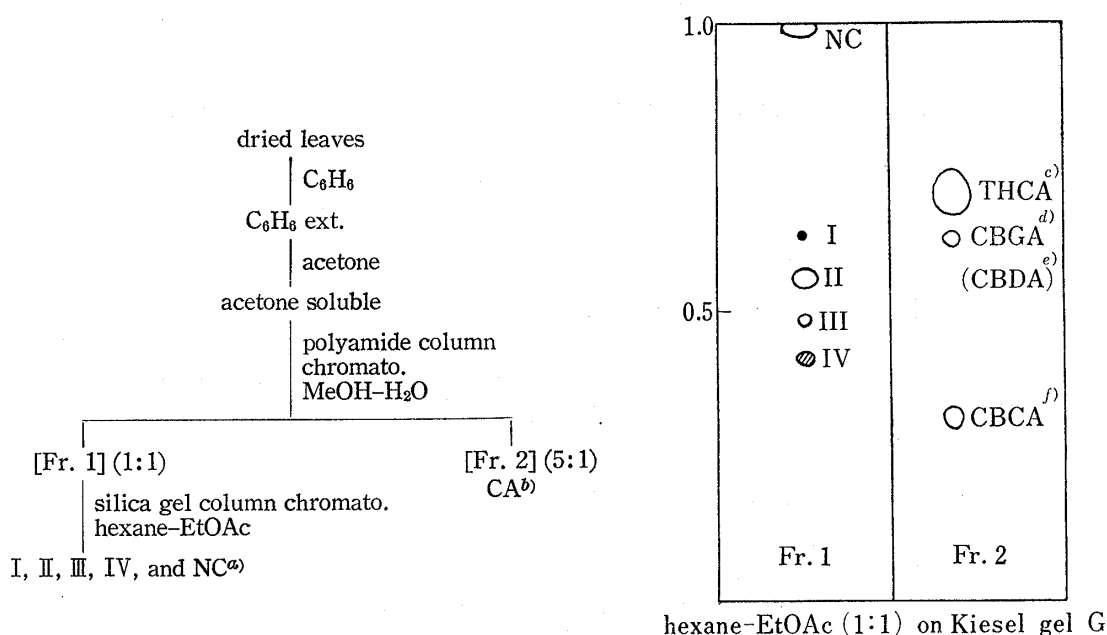


Fig. 1. Extraction, Purification and TLC of Spiro-compounds

- 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) collapsed 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.

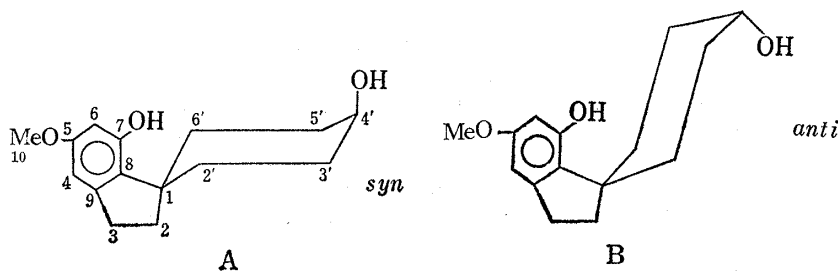


Fig. 2. Possible Structure of Cannabispirol (IV)

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), $\text{C}_{15}\text{H}_{20}\text{O}_3$, 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, $J=w\text{ h}/2=20\text{ Hz}$), 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 results⁹⁾ as shown in Fig. 3. On the other hand, CMR data of IV' explain that the hydroxyl group should be *anti* against indan skeleton.

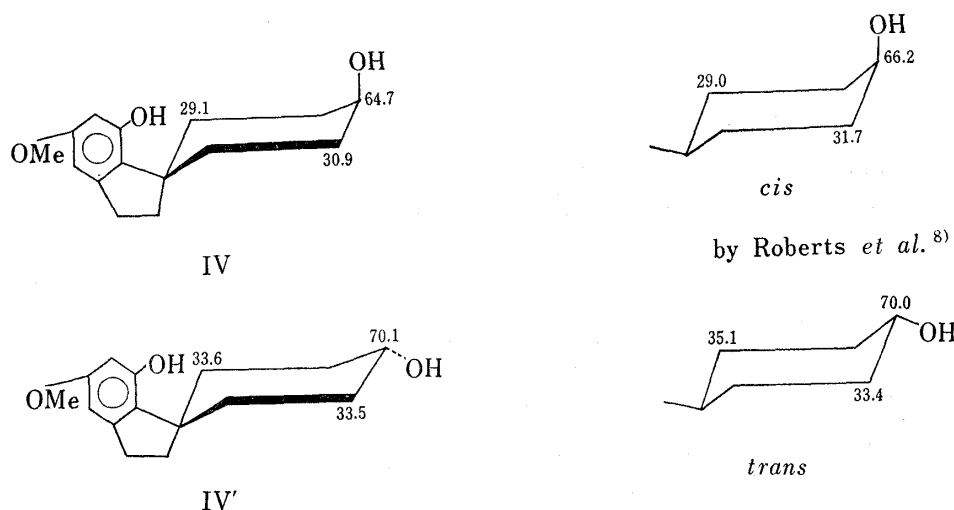


Fig. 3. Stereostructure of Cannabispirol (IV)

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

Compound I (acetyl cannabispirol), colorless needles, $\text{C}_{17}\text{H}_{22}\text{O}_4$, 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 acetoxy signal (2.10 δ), one acetoxymethine signal (5.04 δ , t, $J=4\text{ Hz}$).

Decoupling experiments provided additional evidence for this substitution pattern. Irradiation of the broad bouplet around at 1.36 δ (2H, $J=14\text{ Hz}$, $\text{C}_{2'}$, $\text{C}_{6'}$ eq-H) changed the sextet centering at 2.44 δ (2H, $J=5, 12, 14\text{ Hz}$, $\text{C}_{2'}$, $\text{C}_{6'}$ ax-H) into double doublet ($J=5, 12\text{ Hz}$). On the other hand, irradiation of the sextet at 2.44 δ collapsed the broad doublet at 1.36 δ to a triplet ($J=5\text{ Hz}$). Furthermore, the irradiation of the multiplet near 1.60–1.92 δ (4H, $\text{C}_{3'}$, $\text{C}_{5'}$ -H) changed the triplet at 5.40 δ (1H, t, $J=4\text{ Hz}$, $\text{C}_{4'}$ -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.

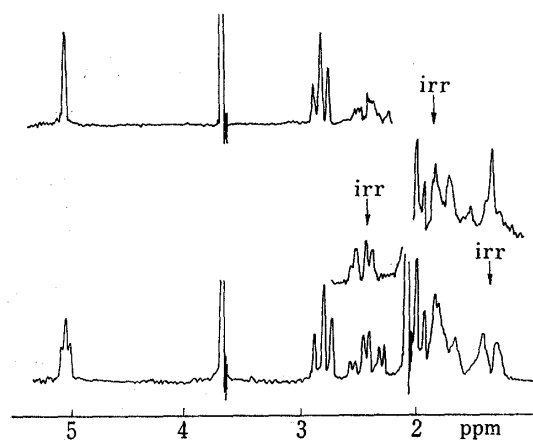


Fig. 4. PMR Spectrum of Acetyl Cannabispirol (I)

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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-H₂O (1:1) to give fraction 1 (containing neutral cannabinoids and spiro-compounds; 10 g) and then eluting with MeOH-H₂O (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°, $[\alpha]_D^{20} +23.6$ ($c=1.21$, CHCl₃). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 273 (3.06), 277 (3.06), 281 (3.08). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3380 (OH), 1728, 1700 (C=O), 1617, 1598 (C=C). PMR (in CDCl₃) 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, C₂-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, C₃-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 (CDCl₃) ppm: 21.4 (OCOCH₃), 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 (OCOCH₃). MS m/e : 290 (M⁺), 230, 215, 202, 189, 176, 161, MS Calcd. for C₁₇H₂₂O₄; 290.152. Found: 290.151.

Fraction 1-2 was recrystallized from CHCl₃-hexane to give II, colorless needles, mp 179—182°. II was identified by the comparison of the physical data of cannabispironone reported (UV, IR, PMR and MS). *Anal.* Calcd. for C₁₅H₁₈O₃: C, 73.14; H, 7.37. Found: C, 72.99; H, 7.35.

Fraction 1-3 was recrystallized from CHCl₃ to give III, mp 170—173°. III was identified by the comparison of the physical data of cannabispironone reported (UV, IR, PMR and MS). *Anal.* Calcd. for C₁₅H₁₆O₃: 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°, $[\alpha]_D^{20} 0^\circ$ ($c=1.12$, MeOH), UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 276.5 (2.93), 280 (2.93), 284 (2.95). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3460, 3280 (OH), 1622, 1601 (C=C), PMR (δ_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, C₂-H), 2.96 (2H, t, $J=7$ Hz, C₃-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, C₇-OH). CMR (δ_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 C₁₅H₂₀O₃: C, 72.35; H, 8.12. Found: C, 72.40; H, 8.21.

Acetylation of IV—IV (30 mg) was acetylated by Ac₂O-pyridine mixture to give crude IVa, which was purified by recrystallization from MeOH to give colorless needles, mp 141—142°. UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 273 (3.29), 280 (3.29). IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1750, 1722 (C=O), 1612, 1585 (C=C). PMR (in CDCl₃) 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, C₂-H), 2.09 (3H, s, OAc), 2.36 (3H, s, OAc), 2.86 (2H, t, $J=6$ Hz, C₃-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 C₁₉H₂₄O₅: 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 H₂O 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 cannabispironone.

Reduction of II by NaBH₄—II (100 mg) was dissolved in MeOH (10 ml) and NaBH₄ (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 H₂O 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 $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 3280, 3150 (OH), 1610, 1596 (C=C). PMR (δ_5 -pyr.) ppm: 1.60—2.00 (4H, m, C_{3'},_{5'}-H), 2.12 (2H, t, $J=7$ Hz, C₂-H), 2.90 (2H, t, $J=7$ Hz, C₃-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, C₇-OH). CMR (δ_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 C₁₅H₂₀O₃; 248.141. Found: 248.141.

Partial Acetylation of IV—IV (20 mg) was dissolved in AcOH (2.5 ml) and BF₃ ether solution (1 ml) was added. The mixture was stirred for 24 hr at room temperature. The reactant was diluted with H₂O 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.

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