CANNABIS XI. SYNTHESIS OF CANNABIGERORCINIC-CARBOXYL-^{14}C ACID, CANNABIGEROVARINIC-CARBOXYL-^{14}C ACID, CANNABIDIVARINIC-CARBOXYL-^{14}C ACID AND dl-CANNABICHROMEVARINIC-CARBOXYL-^{14}C ACID

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SUMMARY

The preparation of cannabigerorcinic-carboxyl-^{14}C acid, cannabigerovarinic-carboxyl-^{14}C acid, cannabidivarinic-carboxyl-^{14}C acid and dl-cannabichromevarinic-carboxyl-^{14}C acid is described. The ^{14}C-label was introduced into the carboxyl carbon via methyl magnesium carbonate-^{14}C.

Key Words: Cannabinoid acid, Cannabigerorcinic acid, Cannabigerovarinic acid, Cannabidivarinic acid, Cannabichromevarinic acid.

RESULTS AND DISCUSSION

Radioactive tetrahydrocannabinol(THC), its derivatives, and cannabinol(CBN) have been synthesized by many groups (1-4) for biological evaluation, especially of the metabolism of THC, the major active constituent of marijuana.

We have already established the biosynthetic pathway of cannabinoid acid (5) on pentyl homologues using mainly cannabigerolic-carboxyl-^{14}C acid and cannabidiolic-carboxyl-^{14}C acid which were synthesized from carbon-^{14}C dioxide by a modified procedure of Mechoulam (6).

We now wish to report the syntheses of cannabigerorcinic-carboxyl-^{14}C acid, cannabigerovarinic-carboxyl-^{14}C acid, cannabidivarinic-carboxyl-^{14}C acid and dl-cannabichromevarinic-carboxyl-^{14}C acid.

As we pointed out (7), when all cannabinoid acids are heated at 150°-160° for 10 min, they are completely decarboxylated to liberate carbon dioxide and corresponding neutral cannabinoids without by-product formation. From this

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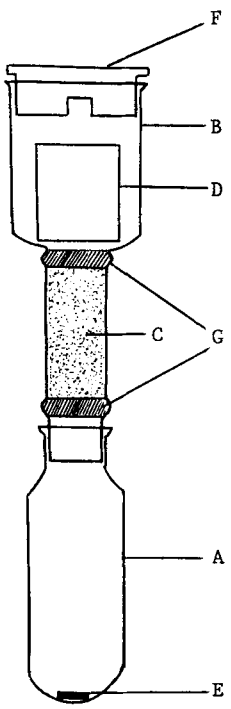
phenomenon, it seems that carboxylation of the neutral cannabinoid is a convenient way to label the carboxyl carbon of cannabinoid acid by ^{14}C.

Mechoulam and his coworkers (6) established the syntheses of resorcinol derivatives by α-carboxylation with methyl magnesium carbonate(MMC) in the large-scale synthesis. We have used their method with radioactive MMC to get radioactive resorcinol derivatives, that is, cannabigerolic-carboxyl-^{14}C acid and cannabidiolic-carboxyl-^{14}C acid (5). In that case, we used a Manifold apparatus (8) for the chemical reaction. However, since the apparatus is occasionally very tricky and is not suitable for a small-scale synthesis, the simple apparatus as shown in Fig.1 was used in this investigation.



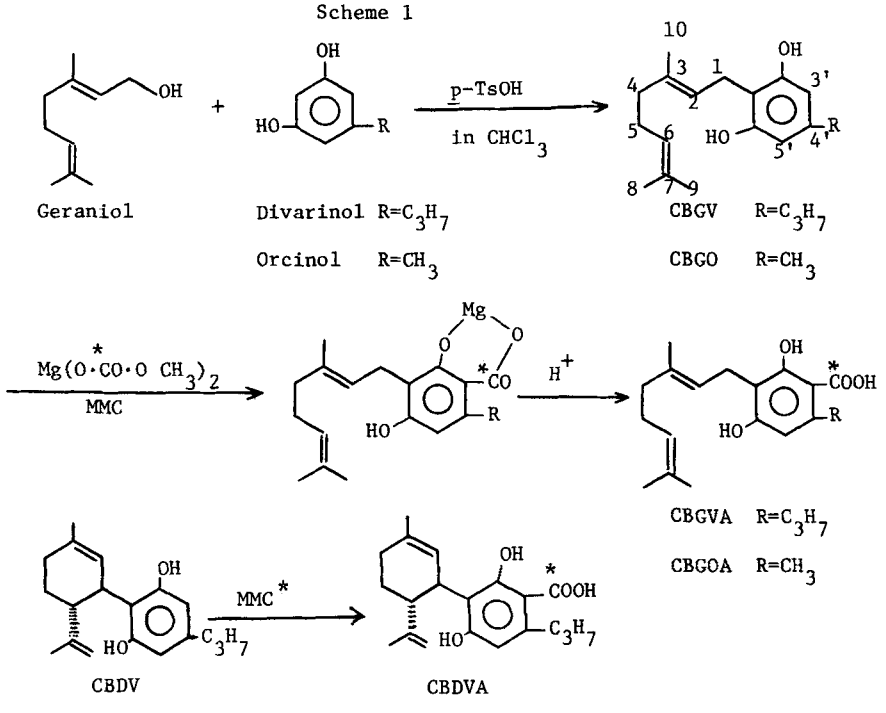
A: Reaction tube-A
5 ml volume, 15/35 outer joint
(1.7 cm x 4 cm)
B: Reaction tube-B, upper section(2.5 cm x 4 cm)
lower section made from 15/35 inner tube
(1.5 cm x 7 cm)
C: $CaCl_2$ anhydrous powder(1.5 cm x 2 cm)
D: $^{14}CO_2$-generation tube(1.5 cm x 2 cm)
E: Stirring magnet
F: Rubber stopper, drilling hole halfway down
G: Glass wool

Fig. 1 Apparatus

After complete evaporation of MeOH, the magnesium methylate was redissolved in freshly distilled N,N-dimethylformamide(DMF). The CO_2-generation tube(D) and rubber stopper(F) were inserted and the apparatus was evacuated briefly through

a hypodermic needle in the stopper(F). After cooling the reaction tube(A) sufficiently and stirring gently, lactic acid was injected into the generation tube (D) through the stopper(F) via a metal syringe and continuously stirred for 1 hr under cooling with an ice-NaCl bath. The reaction tube(A) was taken off from(B) and dried cannabigerorcin(CBGO), cannabigerovarin(CBGV) (9) or cannabidivarin (CBDV) (9) was added to the MMC-DMF solution and then heated at 120° for 2 hr. After cooling, the reaction mixture was adjusted to pH 2 and extracted repeatedly with $CHCl_3$-MeOH. The crude cannabinoid acid was purified by column chromatography over silica gel using hexane-EtOAc as a solvent and crystallized from a hexane-$CHCl_3$ mixture to yield a pure compound as colorless crystals which was identified by direct comparison(mixed mp and thin-layer chromatography(TLC)) with an authentic sample (10). This reaction is summarized in Scheme 1.

Scheme 1

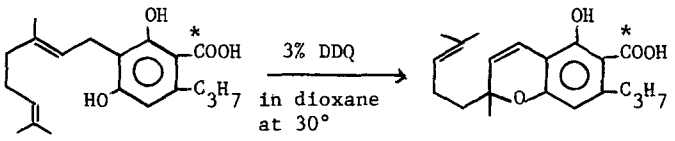


The determination of the radioactive position in the individual material was confirmed by decarboxylation back to the corresponding non-radioactive neutral

cannabinoid.

Concerning the synthesis of labeled cannabichromevarinic acid, the chromene moiety generally has been prepared by the cyclization of the terpenoid with 2,3-dichloro-3,5-dicyano-p-benzoquinone(DDQ) in boiling benzene (11, 12) or by the condensation of the terpenaldehyde and resorcinol derivatives with pyridine (13, 14). However, since both reactions are carried out at high temperatures, it is impossible to use these reactions for the syntheses of cannabinoid acids because they easily decarboxylate. Moreover, it seems that cannabichromevarinic acid(CBCVA), as well as cannabichromenic acid(CBCA), is a fragile compound with respect to high temperature (15), light (7) or acidic medium (16). Thus, we should choose a mild reaction.

Scheme 2



CBGVA dl-CBCVA

On continuing investigation of cyclization of cannabigerovarinic acid (CBGVA) with DDQ, it became evident that CBGVA is converted into dl-CBCVA in good yield only when 3% DDQ-dioxane solution was used at 30°. After 1 hr reaction and evaporation of the solvent, the crude product was redissolved in $CHCl_3$-MeOH and washed according to Folch's method (17). The crude dl-cannabichromevarinic-carboxyl-^{14}C acid, from which DDQ was almost completely removed by this washing, was purified by preparative TLC on silica gel using hexane-EtOAc and $CHCl_3$-MeOH-H_2O as solvents to give pure dl-cannabichromevarinic-carboxyl-^{14}C acid which was identified by comparison with an authentic sample. The specific activity was determined by gas liquid chromatography(GLC).

Biosynthetic and metabolic studies using the above radioactive cannabinoid acids are in progress and the details will be reported elsewhere.

EXPERIMENTAL

Melting points were taken on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were determined with a Hitachi 124 Spectrophotometer. Infrared(IR) spectra were obtained with a Nihon Bunko Model DS-301 Spectrophotometer. Nuclear Magnetic Resonance(NMR) spectra were taken in $CDCl_3$ solution at 100 MHz on a JEOL PS-100 Spectrometer and chemical shifts are given in ppm with tetramethylsilane as internal standard, and signal multiplicities are represented by s (singlet), d(doublet), t(triplet) and m(multiplet). Mass spectra were taken on a JEOL-JMS-OI SG. GLC was conducted under the following conditions: Shimadzu Gas Chromato GC-4BM with 1.5% OV-1(2m x 3 mm), column temperature 220°, detector temperature 245°, carrier gas; N_2 40 ml/min, H_2 80 ml/min, air 500 ml/min. Thin-layer plates were prepared with Kieselgel G(Merck) and developed in the following solvent systems: hexane-EtOAc(1:2), $CHCl_3$-MeOH-H_2O(30:10:1), benzene-MeOH-AcOH (45:8:4)(for cannabinoid acids); benzene, hexane-benzene-diethylamine(20:10:1) (for neutral cannabinoids). Column chromatography was carried out with Kieselgel 60(0.06-0.2 mm, Merck) using 70-100 times the quantity of the material. Radiochromatography was detected by TLC scanner(Aloka Model TRM-1B). Radioactivity was measured by a liquid scintillation spectrometer(Packard TRI-Carb Model 3375) using the scintillator mixture(POP 4g, diethyl POPOP 0.1 g, EtOH 50 ml and toluene 950 ml).

Synthesis of Cannabigerorcin----- Orcinol(2g, Merck) and geraniol(3g, Wako Pure Chemical Industries) were dissolved in $CHCl_3$(400 ml) containing p-toluenesulfonic acid(80 mg) and stirred at room temperature for 12 hr in the dark. The reaction mixture was washed with saturated $NaHCO_3$ solution and then repeatedly with H_2O. The $CHCl_3$ was evaporated in vacuo to give a syrup(3.5 g). The crude product was purified on silica gel(200 g) column chromatography using benzene as a solvent to give CBGO(780 mg) which was recrystallized from hexane-$CHCl_3$ mixture to give colorless prisms, mp 59°. Anal. Calcd. for $C_{17}H_{24}O_2$: C, 78.42; H, 9.29. Found: C, 78.32; H, 9.18. UV λ_{max}^{MeOH} nm(logε): 270(2.97), 277(2.95), 281(2.93), IR ν_{max}^{KBr} cm^{-1}: 3400, 3250(OH), 1633, 1587(C=C). NMR($CDCl_3$) δ: 1.60, 1.68, 1.80(3H x 3, each s,

$C_{8,9,10}$-CH_3), 2.20(3H, s, $C_{4'}$-CH_3), 3.38(2H, d, J=10.5 Hz, C_1-CH_2), 4.90-5.40 (2H, m, $C_{2,6}$-H), 6.23(2H, s, $C_{3',5'}$-H). Mass m/e: 260(M^+), 191, 175, 163, 143, 137 and 123.

Synthesis of Cannabigerorcinic acid----- Mg(38 mg) was refluxed in absolute MeOH (2ml) for 1 hr and then the MeOH was evaporated completely. Magnesium methylate was redissolved in freshly distilled DMF(0.2 ml). The CO_2-generation tube, which contained $BaCO_3$(230 mg), and the rubber stopper were inserted and the apparatus was evacuated. The DMF solution was chilled with an ice-NaCl bath under stirring. Lactic acid(5 ml, 50%) was injected into the CO_2-generation tube through the stopper and continuously stirred for 1 hr. The reaction tube-A was taken off from the reaction tube-B and dried CBGO(100 mg) was added to the MMC-DMF solution and then heated at 120° for 2 hr. After cooling, the reaction mixture was adjusted to pH 2 with dilute HCl and extracted repeatedly with $CHCl_3$-MeOH(2:1). The crude cannabigerorcinic acid(CBGOA) was purified by column chromatography over silica gel(8 g) using hexane-EtOAc(1:2) as a solvent to give CBGOA(14 mg) which was recrystallized from hexane-$CHCl_3$, colorless prisms, mp 128-130°, Anal. Calcd. for $C_{18}H_{24}O_4$: C, 71.02; H, 7.95. Found: C, 70.89; H, 7.95. UV λ_{max}^{MeOH} nm(logε): 221 (4.39), 257(3.92), 299(3.47), IR ν_{max}^{KBr} cm^{-1}: 3540(OH), 1640(C=O), 1625, 1580(C=C). NMR($CDCl_3$) δ: 1.60, 1.70, 1.84(3H x 3, each s, $C_{8,9,10}$ -CH_3), 2.80(4H, d, $C_{4,5}$ -CH_2-CH_2), 2.58(3H, s, $C_{4'}$-CH_3), 3.45(2H, d, J=8 Hz, C_1-CH_2), 5.06(1H, broad s, C_2-H), 5.28(1H, t, J=6 Hz, C_6-H), 5.60-6.40(1H, broad s, $C_{6'}$-OH), 6.26(1H, s, $C_{5'}$-H), 11.78(1H, s, $C_{2'}$-OH).

Synthesis of Cannabigerorcinic-carboxyl-^{14}C acid----- CBGO(50 mg) was added to MMC-^{14}C-DMF solution which was produced from $Ba^{14}CO_3$(160 mg, 2 mCi, New England Nuclear) and heated at 120° for 2 hr. The reaction mixture was adjusted to pH 2 and extracted and then washed until neutral by Folch's method (17) as previously done. The crude procuct was purified by column chromatography on silica gel eluting with hexane-EtOAc(1:2)(each fraction was 2 ml) as previously done. Fractions 19-33 were pooled and the solvent was evaporated *in vacuo* to give cannabigerorcinic-carboxyl-^{14}C acid(4.1 mg, 7.64 x 10^7 dpm; 2.55 mCi/mmol) which was crystallized

from a small amount of hexane-$CHCl_3$, colorless prisms, mp 128-130°, and which was identified by comparison(mixed mp and TLC) with an authentic sample. The radiochemical purity was confirmed by a TLC scanner.

Synthesis of Cannabigerovarinic-carboxyl-^{14}C acid----- CBGV(50 mg) was reacted and the product was purified by the same procedure as cannabigerorcinic-carboxyl-^{14}C acid. Fractions 19-25 were pooled and the solvent was evaporated *in vacuo* to give cannabigerovarinic-carboxyl-^{14}C acid(3.4 mg, 5.58 x 10^7 dpm; 2.54 mCi/mmol), mp 66-68°, which was identified by comparison(mixed mp and TLC) with an authentic sample (10). The radiochemical purity was confirmed by TLC scanner.

Synthesis of Cannabidivarinic-carboxyl-^{14}C acid----- CBDV(20 mg) was reacted and the product was purified as done previously. Fractions 9-27 were pooled and the solvent was evaporated to give cannabidivarinic-carboxyl-^{14}C acid(4.1 mg, 6.79 x 10^7 dpm; 2.47 mCi/mmol), mp 102-105° which was identified by comparison(mixed mp and TLC) with an authentic sample (10). The radiochemical purity was confirmed by TLC scanner.

Synthesis of dl-Cannabichromevarinic-carboxyl-^{14}C acid----- Cannabigerovarinic-carboxyl-^{14}C acid(2.79 x 10^6 dpm) was dissolved in dioxane(0.1 ml) containing DDQ(3 mg) and then stirred at 30° for 1 hr in the dark. The solvent was evaporated under nitrogen flow and the residue was redissolved in $CHCl_3$-MeOH(2:1)(2 ml). After washing using a modification of Folch's method (17) until the $CHCl_3$ layer became almost colorless, the crude product was purified by silica gel TLC using hexane-EtOAc(1:2) and $CHCl_3$-MeOH-H_2O(30:10:1) to give pure dl-cannabichromevarinic-carboxyl-^{14}C acid(1.78 x 10^6 dpm) which was identified by direct comparison with an authentic sample (10). The specific activity was determined by GLC. Calibration curve was prepared by using cannabichromevarin(CBCV) (9). Area and weight in individual concentrations of CBCV are as follows: Weight(μg): 0.096, 0.193, 0.372, 0.743; Area(mm^2): 14.1, 34.2, 69.0, 136.0. Cannabichromevarinic-carboxyl-^{14}C acid was dissolved in MeOH(0.3 ml) and 1 μl was injected. Area 48.0 mm^2; dl-cannabichromevarinic-carboxyl-^{14}C acid, 0.082 mg, 3.24 mCi/mmol. The radiochemical purity was confirmed by TLC scanner.

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