Synthesis and Antimicrobial Activities of Certain Cannabichromene and Cannabigerol Related Compounds

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Abstract □ Cannabichromene homologs, analogs, and isomers as well as the C_1-homolog and isomer of cannabigerol were prepared and tested for their antimicrobial and antifungal properties. Spectral data of all compounds synthesized are presented.

Keyphrases □ Cannabichromene—analogs, synthesis and antimicrobial activities of related compounds, cannabigerol □ Antimicrobial activities —cannabichromene and cannabigerol, analogs, synthesis and related compounds □ Analogs—cannabichromene and cannabigerol, synthesis and antimicrobial activities of related compounds

Different cannabinoids have been isolated from *Cannabis sativa* L. (1). Most of the biological studies carried out concentrate on the major psychoactive constituent Δ^9-tetrahydrocannabinol and its isomers (2–4). However, little attention has been given to the biological activity of cannabichromene (I) because of its relatively low concentration in the plant material.

Recently, a procedure was developed for the synthesis of I in high yield (60%) (5), which made the material available for pharmacological testing.

The biological activities of cannabichromene has been reported (6–9), as well as its homologs and isomers (10, 11). This paper describes the synthesis and antimicrobial activities of cannabichromene homologs, analogs, and isomers and that of cannabigerol-C_1, isocannabigerol-C_1, and tetrahydrocannabigerol-C_1.

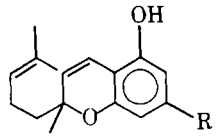
EXPERIMENTAL

Melting points[1] were determined in open glass capillary tubes and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra[2] were recorded in deuterochloroform or in deuteromethanol using tetramethysilane as the internal standard. IR spectra[3] were recorded in liquid film, chloroform, or in potassium bromide pellets. Mass spectra[4] were recorded at an electron energy of 70 eV. UV spectra[5] were recorded in methanol. ^{13}C-NMR spectra[6] were recorded in deuterochloroform or deuteromethanol.

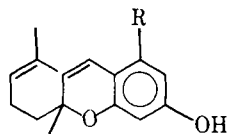
Biological Activities–Antibacterial and Antifungal Activities—All compounds were tested for activity against Gram-positive, Gram-negative, and acid-fast bacteria and selected fungi. A qualitative screen was performed on all compounds, while quantitative assays were done on active compounds only. Routine qualitative screens were carried out using the agar well diffusion assay as previously described (12). Minimum inhibitory concentrations were determined using the twofold (broth) serial dilution method (12) with a concentration of 100 μg/ml in the first tube. Streptomycin sulfate was used as a positive control for antibacterial activity, while amphotericin B was used as an antifungal positive control.

2-Methyl-2-(4′-methylpent-3′-enyl)-5-hydroxy-7-methylchromene (II)—Orcinol (3.45 g, 0.028 mole) was dissolved in 55 ml of toluene with heating and stirring. Equimolar quantities of *tert*-butylamine were added to the resulting solution followed by dropwise addition of citral. The reaction mixture was refluxed for 9 hr. The mixture was then cooled to room temperature, transferred to a round-bottom flask, and the solvent evaporated. GC analysis of the reaction mixture showed 48.3% conversion to II. About one half of the crude reaction product (5 g) was applied over a dry packed silica gel 60 column (100 g) using 15 ml of cyclohexane–chloroform (1:1), and elution was continued with the same solvent. Fractions of 25 ml were collected and combined based on TLC similarities. Fractions containing II (1.96 g) were rechromatographed as before to yield II of >95% purity as light brown oil. UV λ_{max} (methanol) nm (log ϵ): 229 (4.38) and 280 (3.92); IR (liquid film) major bands at 3400, 2970, 2820, 1620, 1575, and 1420 cm^{-1}; ^{1}H-NMR ($CDCl_3$) signals at δ 6.68 (1H, d, J = 10 Hz), δ 6.27 (1H, s), δ 6.13 (1H, s), δ 5.45 (1H, d, J = 10 Hz), δ 5.12 (1H, br, t, J = 6 Hz), δ 2.17 (3H, s), δ 1.67 (3H, s), δ 1.58 (3H, s), and δ 1.38 (3H, s); ^{13}C-NMR ($CDCl_3$) signals at δ 154.1 (s), δ 151.3 (s), δ 139.6 (s), δ 131.5 (s), δ 127.1 (d), δ 124.4 (d), δ 117.9 (d), δ 109.9 (d), δ 108.8 (d), δ 107.2 (s), δ 74.4 (s), δ 41.1 (t), δ 26.2 (q), δ 25.5 (q), δ 22.7 (t), δ 21.4 (q), and δ 17.5 (q); MS M$^+$ at m/z: 258 (10%), for $C_{17}H_{22}O_2$, 175 (100%) 243 (3%), 215 (7%), 183 (3%).

2-Methyl-2-(4′-methylpent-3′-enyl)-5-methyl-7-hydroxychromene (III)—Compound III was formed as a side product from the synthesis of II when pyridine was used both as a base and a solvent. Equi-

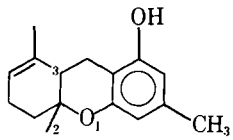


I R = C_5H_{11}
II R = CH_3
VI R = H

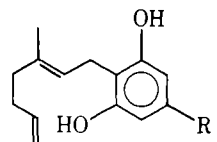


III R = CH_3
VII R = H
III R = C_5H_{11}

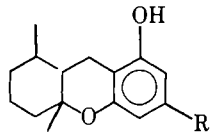
VIII R = $(CH_2)_7$—CH=CH—$(CH_2)_5$—CH_3



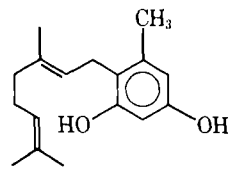
IV



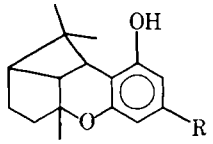
X R = CH_3, XIII R = C_5H_{11}



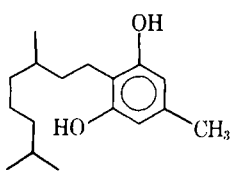
V R = CH_3 V′ R = C_5H_{11}



XI



IX R = OH
XVII R = C_5H_{11}



XII

[1] Thomas Hoover Unimelt.
[2] JEOL C-60HL.
[3] Perkin-Elmer 267.
[4] Finnigan 3200, MS/DS system.
[5] Beckman Acta III.
[6] JEOL FX-60 operating at 15.03 MHz.

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molar quantities of pyridine and citral were added to 1.73 g of (0.014 mole) orcinol. The mixture was refluxed with stirring for 7 hr, cooled, and evaporated to yield a brownish residue (3.97 g). On TLC[7], the residue showed two major spots using chloroform–cyclohexane (4:1) and benzene–hexane (4:1) as solvent systems. The crude reaction mixture was purified on a silica gel 60 column followed by preparative chromatography[8] using benzene–hexane (4:1) as the solvent system. The compound with the high R_f value (0.30, 15%) was II, while that with the low R_f value (0.16, 24%) was identified as III. Compound III was isolated as an amber colored oil. UV λ_{max} (methanol) nm (log ϵ): 224 (4.17), 285 (3.70), and 305 (3.57); IR (liquid film) major bands at 3380, 2970, 2915, 1610, and 1460 cm^{-1}; ^{1}H-NMR ($CDCl_3$) signals at δ 6.47 (1H, d, J = 9 Hz), δ 6.18 (2H, s), δ 5.43 (1H, d, J = 9 Hz), δ 5.12 (2H, br, t, J = 7 Hz), δ 2.18 (3H, s), δ 1.67 (3H, s), δ 1.58 (3H, s), and δ 1.37 (3H, s); ^{13}C-NMR ($CDCl_3$) signals at δ 156.1 (s), δ 154.7 (s), δ 135.1 (s), δ 131.4 (s), δ 126.5 (d), δ 124.4 (d), δ 119.6 (d), δ 113.2 (s), δ 109.6 (d), δ 101.7 (d), δ 78.1 (s), δ 41.1 (t), δ 26.2 (q), δ 25.5 (q), δ 22.7 (t), δ 18.3 (q), and δ 17.5 (q); MS M^+ at m/z 258 (6%) for $C_{17}H_{22}O_2$, 175 (100%).

2-Methyl-2-(4′-methylpent-3′-enyl)-5-hydroxy-7-methylchroman (IV) and 2-Methyl-2-(4′-methylpentyl)-5-hydroxy-7-methylchroman (V)—Compounds IV and V were prepared by catalytic hydrogenation of Compound II. A mixture of IV and V was formed when the reaction was allowed to go for 20 min under hydrogen atmosphere. However, Compound V was the only product obtained when hydrogenation was allowed to occur for a longer period of time or under pressure (0.702 kg/cm^2). Compound II (0.15 g, 5.8×10^{-4} mole) was dissolved in 8 ml of ethanol and 20 mg of 5% pallidium on carbon (Pd/C) was added. The reaction mixture was stirred for 20 min under a hydrogen atmosphere. TLC examination of the reaction product on silver nitrate-treated silica gel plates using chloroform–benzene (8:2) as the solvent system showed two major spots with R_f 0.22 and 0.35. The two compounds were separated on an HPLC using a reversed-phase column[9] with methanol–water (8:2) as the solvent system. Twenty-two milligrams (14.6%) of IV and 99 mg (66%) of V were obtained. Compound IV was isolated as a light yellow oil. UV λ_{max} (methanol) nm (log ϵ): 283 (2.92), 273 (2.92), 232 (3.82), 217 (3.95). IR ν_{max} ($CHCl_3$) cm^{-1}: 2968, 2924, 2858, 1620, 1580, 1450, 1375, 1349, 1320, 1260, 1155, 1132, 1100, 1055, 995, 875; ^{1}H-NMR ($CDCl_3$) δ 6.27 (1H, s, aromatic H), δ 6.17 (1H, s, aromatic H), δ 5.15 (1H, t, olefinic proton), δ 2.61 (2H, t, benzylic proton), δ 2.18 (3H, s, aromatic methyl), δ 1.67 (3H, s, methyl on double bond), δ 1.60 (3H, s, methyl on double bond), δ 1.28 (3H, s, methyl on oxygenated carbon); MS M^+ at m/z 260 (25.5%) for $C_{17}H_{24}O_2$, 177 (36%), 175 (55%), 136 (100%). The tetrahydro-derivative was separated as a light yellow oil; UV λ_{max} (methanol) nm (log ϵ): 282 (2.93), 275 (2.93), 233 (3.88), 217 (4.09); IR ν_{max} ($CHCl_3$) cm^{-1}: 2950, 1622, 1580, 1450, 1378, 1350, 1320, 1260, 1150, 1130, 1100, 1070, 995, 875; ^{1}H-NMR ($CDCl_3$): δ 6.16 (1H, s, aromatic H), δ 6.03 (1H, s, aromatic H), δ 2.56 (2H, t, benzylic protons), δ 2.16 (3H, s, aromatic methyl), δ 1.25 (3H, s, methyl on oxygenated carbon), δ 0.92 (3H, d, J = 6 Hz), and δ 0.80 (3H, d, J = 6 Hz); MS M^+ 262 (36.5%) for $C_{17}H_{22}O_2$, 177 (57%), and 136 (100%).

2-Methyl-2-(4′-methylpent-3′-enyl)-5-hydroxychromene (VI) and 2-Methyl-2-(4′-methylpent-3′-enyl)-7-hydroxy-chromene (VII)—Compounds VI and VII were prepared by reaction of 1.53 g of resorcinol (0.014 mole), and equimolar amounts of *tert*-butylamine and citral in 27.5 ml of toluene following the same conditions described under preparation of II. The reaction of the nonsubstituted resorcinol under these conditions result in the formation of both VI and VII. The crude reaction mixture was purified by repeated chromatography on a silica gel 60 column and preparative liquid chromatography[10] with benzene as the solvent to obtain Compounds VI and VII in 10% yield. Compound VI was isolated as a yellowish-brown oil. UV λ_{max} (methanol) nm (log ϵ): 225 (4.35) and 278 (3.90); IR (liquid film) major bands at 3395, 2970, 2920, 1610, 1580, and 1460 cm^{-1}; ^{1}H-NMR ($CDCl_3$) signals at δ 6.38 (1 H, d, J = 10 Hz), δ 5.52 (1H, d, J = 10 Hz), δ 5.13 (1H, br, t, J = 6 Hz), δ 1.67 (3H, s), δ 1.60 (3H, s) and 1.40 (3H, s); ^{13}C-NMR ($CDCl_3$) signals at δ 154.2 (s), δ 151.5 (s), δ 131.5 (s), δ 129.0 (d), δ 128.1 (d), δ 124.4 (d), δ 117.0 (d), δ 109.7 (s), δ 109.1 (d), δ 107.9 (d), δ 78.4 (s), δ 41.4 (t), δ 26.2 (q), δ 25.5 (q), δ 22.7 (q), δ 17.5 (q); MS m/z (%), M^+ 244 (2%), 161 (100%), 115 (2%). Compound VII was separated as a brownish oil; UV λ_{max} (methanol) nm (log ϵ): 220 (4.39), 283 (3.86), 305 (3.83), and 310 (sh, 3.79); IR (liquid film) major bands at 3400, 2970, 2920, 1650, and 1500 cm^{-1}; ^{1}H-NMR ($CDCl_3$) signals at δ 6.68 (1H, d, J = 9 Hz), δ 6.25 (2H, br, s), δ 6.07 (1H, br, s), δ 5.30 (1H, d, J = 9 Hz), δ 5.03 (2H, br, t, J = 6 Hz), δ 1.63 (3H, s), δ 1.53 (3H, s), and δ 1.18 (3H, s); ^{13}C-NMR ($CDCl_3$) signals at δ 156.8 (s), δ 154.5 (s), δ 131.6 (s), δ 127.3 (d), δ 126.7 (d), δ 124.4 (d), δ 122.5 (d), δ 114.7 (s), δ 107.8 (d), δ 103.8 (d), δ 78.9 (s), δ 41.5 (t), δ 26.6 (q), δ 25.6 (q), δ 22.8 (t), and 17.6 (q); MS M^+ at m/z 244 (6%), 161 (100%), and 115 (7%).

2-Methyl-2-(4′-methylpent-3′-enyl)-5-hydroxy-7-pentadec-8″enylchromene (VIII)—Compound VIII was prepared by reaction of 5-pentadec-8′-enyl resorcinol with citral in the presence of *tert*-butylamine (equimolar amounts) in toluene solution following the same procedure as described under synthesis of I. Pentadecenyl resorcinol was isolated from *Ginkgo* fruits. Compound VIII was isolated as a brownish colored oil (60% yield). UV λ_{max} (methanol) nm (log ϵ): 228 (4.39) and 279 (3.97); IR (liquid film) major bands at 3400, 2920, 2855, 1620, 1575, 1430 cm^{-1}; ^{1}H-NMR ($CDCl_3$) signals at δ 6.62 (1 H, d, J = 10 Hz), δ 6.22 (1H, s), δ 6.10 (1H, s), δ 5.42 (1H, d, J = 10 Hz), δ 1.65 (3H, s), δ 1.57 (3H, s), δ 1.39 (3H, s), δ 1.32 (18H, br, s); ^{13}C-NMR ($CDCl_3$) signals at δ 154.3 (s), δ 151.5 (s), δ 144.8 (s), δ 131.4 (s), δ 130.1 (d), δ 127.2 (d), δ 124.6 (d), δ 117.1 (d), δ 109.2 (d), δ 108.0 (d), δ 107.3 (s), δ 78.3 (s), δ 41.3 (t), δ 36.1 (t), δ 31.9 (t), δ 31.0 (t), δ 29.9 (t, 2C), 29.4 (t, 2C), 29.0 (t), δ 27.4 (t, 3C), δ 26.3 (q), δ 25.6 (q), δ 22.9 (t), δ 22.7 (t), δ 17.6 (q), δ 14.1 (q); MS M^+ at m/z 452 (0.16%), for $C_{31}H_{48}O_2$, 369 (100%), 187 (16%), 174 (11%).

2-Methyl-2-(4′-methylpent-3′-enyl)-5,7-dihydroxychromene—Phloroglucinol (2.52 g, 0.02 mole) was dissolved in 25 ml of acetonitrile–toluene (1:1). *tert*-Butylamine (0.022 mole) was added to the resulting solution, followed by the dropwise addition of 0.022 mole citral, and the reaction mixture was refluxed for 1 hr. The material was applied on a silica gel 60 column packed in 20% ethyl acetate–cyclohexane; 220 mg of the cyclol (IX) were obtained. Yield: 4.4%.

The cyclol was obtained as fine needles (cyclohexane–acetone), mp 150–150.5°. UV λ_{max} (methanol) nm (log ϵ): 280 (2.11), 240 (3.06), 222 (3.61); IR ν_{max}^{KBr} cm^{-1}: 3360, 2970, 2920, 1615, 1596, 1490, 1462, 1378, 1365, 1340, 1312, 1270, 1255, 1240, 1215, 1160, 1150, 1135, 1122, 1081, 1065, 1032, 995, 945, 900, 878, 825, 750; ^{1}H-NMR ($CDCL_3$) signals at: δ 5.93 (2H, s, aromatic), δ 2.76 (1H, m, C_3—H), δ 1.46, δ 1.35, and δ 1.01 (3H, each, s); ^{13}C-NMR: δ 157.82 (s), δ 157.32 (s), δ 155.94 (s), δ 109.42 (s), δ 98.50 (d), δ 96.94 (d), δ 84.33 (s), δ 47.04 (d), δ 37.5 (t), δ 35.54 (t), δ 29.76 (q), δ 29.04 (d or q), δ 28.00 (d), δ 23.7 (q), δ 22.3 (q or d); MS M^+ 260 (8%), 245 (3%), 217 (6%), 189 (7%), 177 (100%).

2-Geranyl-5-methyl Resorcinol (X) and 4-Geranyl-5-methyl Resorcinol (XI)—Compound X was prepared by condensation of geraniol (3.47 ml) and orcinol (2.844 g, 20 mmoles) in methylene chloride (100 ml) in the presence of *p*-toluenesulfonic acid (20 mg) at 20° for 30 min. The reaction mixture, after workup, was repeatedly chromatographed on silica gel to yield X (794 mg, 15%) and XI (100 mg, 2%). Compound X was obtained as a crystalline material, mp 50–51° (hexane). UV λ_{max} (methanol) nm (log ϵ): 275 (2.93), 209 (4.55); IR ν_{max}^{KBr} (cm^{-1}): 3420, 3380, 3268, 3120, 2960, 2908, 2850, 1630, 1580, 1510, 1450, 1375, 1322, 1268, 1220, 1198, 1145, 1080, 1042, 985, 825; ^{1}H-NMR ($CDCL_3$) δ 6.2 (2H, s), δ 5.36–5.08 (2H, m, olefinic), δ 3.4 (2H, d, J = 8 Hz), δ 2.18 (3H, s), δ 1.81 (3H, s), δ 1.70 (3H, s) and δ 1.60 (3H, s); ^{13}C-NMR: signals at δ 155.0 (s), δ 138.9 (s), δ 137.6 (s), δ 132.0 (s), δ 124.0 (d), δ 122.0 (d), δ 111.0 (d), δ 109.4 (d), δ 39.8 (t), δ 26.6 (t), δ 25.6 (q), δ 22.3 (t), δ 21.0 (t), δ 17.7 (q), δ 16.2 (q); MS M^+ 260 (2.68%) for $C_{17}H_{24}O_2$, 191 (8%), 175 (23%), 163 (22%), 149 (20%), 137 (100%).

Compound XI yielded fine needles, mp 50–51° (hexane). UV λ_{max} (methanol) nm (log ϵ): 283 (3.19), 207 (4.32); IR ν_{max}^{KBr} cm^{-1}: 3200, 2982, 2960, 2830, 1610, 1510, 1470, 1450, 1375, 1335, 1310, 1220, 1140, 1050, 980, 900, 835; ^{1}H-NMR ($CDCl_3$) signals at δ 6.23 (2H, s), δ 5.6–5.13 (2H, m, olefinic), δ 3.3 (2H, d, J = 8 Hz), δ 2.2 (3H, s), δ 2.03 (4H, br, s), δ 1.76 (3H, s), δ 1.66 (3H, s), δ 1.60 (3H, s); ^{13}C-NMR ($CDCl_3$) δ 155.4 (s), δ 154.3 (s), δ 138.8 (s), δ 137.2 (s), δ 131.8 (s), δ 124.2 (d), δ 122.5 (d), δ 118.6 (d), δ 110.1 (d), δ 101.4 (d), δ 39.8 (t), δ 26.7 (t), δ 25.7 (q), δ 25.1 (t), δ 19.9 (q), δ 17.7(q), δ 16.2 (q); MS M^+ 260 (2.92%) for $C_{17}H_{24}O_2$, 191 (13.5%), 175 (26.64%), 163 (17.44%), 149 (22.52%), and 137 (100%).

2-Tetrahydrogeranyl-5-methyl Resorcinol (XII)—Compound X was dissolved in 8 ml of ethanol, 20 mg of 5% Pd/C was added, and the reaction mixture was allowed to proceed under a hydrogen atmosphere for 4 hr. TLC examination of the reaction product showed complete conversion of the starting material. Filtration followed by crystallization from hexane yielded a crystalline material, mp 84–85°. UV λ_{max} (methanol) nm (log ϵ): 280.5 (3.05), 271 (3.09), 232 (sh, 3.00), 209 (4.60); IR λ_{max}^{KBr} cm^{-1}: 3420, 3300, 2960, 2928, 2870, 2850, 1630, 1580, 1518, 1468, 1380, 1365, 1328, 1270, 1200, 1160, 1112, 1025, and 830; ^{1}H-NMR (CD_3OD) signals at δ 6.13 (2H, s), δ 2.56 (2H, t), δ 1.53–1.23 (m, 9H), δ 0.9 (6H, s), δ 0.82 (3H, s); ^{13}C-NMR (CD_3OD): signals at δ 156.9 (s), δ 136.8 (s), δ 114.9 (s), δ 108.7 (d), δ 40.5 (t), δ 38.3 (t), δ 37.4 (t), δ 34.11 (d), δ 29.0 (t), δ 25.7

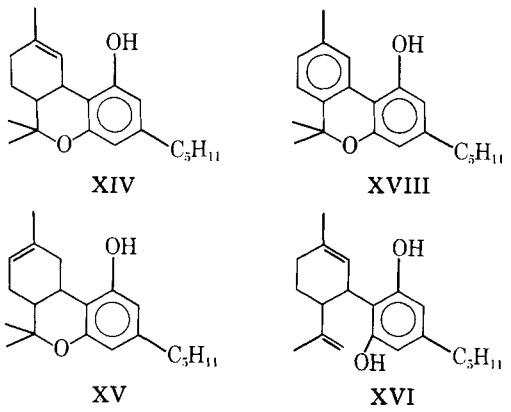
[7] Precoated silica gel G Machery Nagel & Co.
[8] Waters LC-500A.
[9] μBondapackC$_{18}$.
[10] Waters prep. LC 500A.

(t), δ 23.0 (q), δ 21.6 (q), δ 21.3 (t), δ 20.3 (q); MS M^+ at *m/z* 264 (16.4%) for $C_{17}H_{28}O_2$, 150 (11.5%) and 137 (100%).

RESULTS AND DISCUSSION

Cannabichromene (I) derivatives were prepared according to a previously published method for the synthesis of cannabichromene (5). That method involves the reflux of equimolar quantities of the properly substituted resorcinol, citral and *tert*-butylamine in toluene for 9 hr. In this way, 48% of II was obtained. However, when pyridine was used as the solvent and base, Compound III was obtained in a 24% yield. Compounds IV and V were obtained through catalytic hydrogenation of II using 5% Pd/C. When the reaction was allowed to proceed for 20 min, a mixture of IV and V was obtained in a yield of 15 and 66%, respectively. When the reaction was carried out for a longer period of time or under pressure V was obtained quantitatively. ^{1}H-NMR of IV shows that the double bond was located in the side chain as shown by the presence of only one olefinic signal at δ 5.15 (1H, t). This is in contrast to the ^{1}H-NMR spectrum of II, which showed two additional signals at δ 6.68 and 5.45 (1H each, d, J = 10 Hz) characteristic of the double bond at Δ^3 of the chromene system. Compounds VI and VII were obtained in a 10% yield after repeated chromatography, while VIII was obtained in ~60% yield. All compounds were purified by column chromatography over silica gel or through preparative liquid chromatography and were characterized by spectral methods including IR, UV, MS, ^{1}H-NMR and ^{13}C-NMR.

Attempts at preparing cannabichromene derivatives having the R group either as COOH, $COOCH_3$, HN—O(=C)—CH_3, NH—SO_2—CH_3 under the same conditions for preparing cannabichromene failed. We can conclude that the R group on the resorcinol moiety has a significant effect on the reactivity of the molecule with citral to give the chromene structure. When the R group was an electron-withdrawing group (—O(=C)—OH, —O(=C)—OCH_3) the reaction did not proceed. When R was an OH group the reaction proceeded rapidly to many products of which <5% was the desired product. Attempts to increase the yield were unsuccessful. When R was a long chain alkyl group, the yield of the desired product was fairly high (~60%). Even when R was CH_3 a 48% yield was obtained. However, when R was H the yield dropped back to ~10% with almost an equal amount of iso-compound being formed, and thus, the selectivity of the reaction was lost.



XIV

XVIII

XV

XVI

In addition to cannabichromene-type compounds, the antibacterial activity of other cannabinoids were investigated. Δ^9-Tetrahydrocannabinol (XIV), Δ^8-tetrahydrocannabinol (XV), cannabidiol (XVI), cannabinol (XVIII), cannabigerol (XIII), and cannabicyclol (XVII) were tested. Only XIII showed significant activity. Thus, the C_1-homolog of cannabigerol and its isomer were synthesized to test their activity against the various organisms with the idea that the C_1-homolog might be more active than the C_5-homolog as is the case with cannabichromene homologs.

Compounds X and XI were prepared according to a method described previously (13) for the preparation of cannabigerol. Although the yield was reported to be 52%, only a 10% isolated yield was obtained.

Organisms utilized in the screens included Gram-positive, Gram-negative, and acid-fast bacteria as well as different types of fungi (Table I).

Compounds I–XII were subjected to the antibacterial antifungal activity screens (Tables II–V). Qualitative screening using the agar well diffusion assay showed that these compounds possess strong antibacterial and mild antifungal properties. These compounds exhibited large zones of inhibition when compared to positive standards at the same concentrations. The minimum inhibitory concentrations for these compounds were determined using selected bacteria and fungi as recorded in Tables IV and V. The organisms selected for the minimum inhibitory concentration determinations were based on the largest zone of inhibition resulting in the qualitative screen (Tables II and III).

Table I—Antimicrobial Screening Using the Agar Well Diffusion Assay

Organism	ATCC No.	Classification
Bacillus subtilis	6633	Gram-positive bacterium
Staphylococcus aureus	6538	Gram-positive bacterium
Escherichia coli	10536	Gram-negative bacterium
Pseudomonas aeruginosa	15442	Gram-negative bacterium
Mycobacterium smegmatis	607	Acid-fast bacterium
Candida albicans	10231	Yeast-like fungus
Saccharomyces cerevisiae	9763	Yeast-like fungus
Aspergillus niger	16888	Filamentous fungus
Trichophyton mentagrophytes	9972	Dermatophyte

Table II—Qualitative Antibacterial Results [a]

Compound	*B. subtilis*		*E. coli*		*S. aureus*		*M. smegmatis*		*Ps. aeruginosa*	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
XV	7	5	1	—	15	15	5	5	—	—
XIV	—	—	2	—	5	5	2	2	—	—
XVI	10	10	—	—	10	10	10	9	—	—
XIII	25	17	—	—	30	30	25	25	—	—
Streptomycin SO_4	10	10	6	6	7	7	20	20	5	5
XII	25	25	3	3	22	22	20	20	1	1
Streptomycin SO_4	10	10	6	6	8	8	20	20	5	5
X	>35	33	—	—	>35	35	>35	>35	2	2
Streptomycin SO_4	10	10	6	5	11	11	23	23	5	5
XI	22	20	2	2	22	19	23	22	2	2
Streptomycin SO_4	No Growth		10	10	11	10	20	20	6	5
IV	8	5	5	5	10	10	15	12	1	1
V	16	9	5	5	>25	>25	>25	>25	4	3
V′	4	4	5	5	13	13	7	7	1	1
IX	1	1	1	1	10	10	7	6	1	1
VIII	—	—	—	—	10	10	5	5	4	4
Streptomycin SO_4	7	8	2	2	9	9	20	22	3	3
VII	10	7	2	2	—	—	6	4	—	—
XVII	—	—	—	—	—	—	3	1	—	—
VI	10	10	3	2	15	15	20	18	3	—
Streptomycin SO_4	11	10	5	5	10	10	20	22	7	6

[a] Antimicrobial activity was recorded as the width (in millimeters) of the inhibition zone measured from the edge of the agar well to the edge of the inhibition zone.

Table III—Qualitative Antifungal Results [a]

Compound	*C. albicans*		*S. cerevisiae*		*A. niger*		*T. mentagrophytes*	
	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
XV	2	2	—	—	—	—	1	1
XIV	2	2	1	1	—	—	3	2
XVI	2	3	3	2	—	—	4	2
XIII	3	2	6	4	—	—	5	2
Amphotericin B	4	4	2	2	—	—	4	2
XII	9	5	>25	20	4	4	>30	25
Amphotericin B	5	3	12	12	2	2	13	13
X	25	25	25	22	1	1	15	13
Amphotericin B	9	7	7	7	2	2	10	10
XI	12	10	20	20	16	10	22	22
Amphotericin B	9	7	11	15	7	3	Little growth	23
IV	4	4	8	8	2	2	No growth	
V	3	3	22	23	3	3	No growth	
V′	2	3	7	7	1	1	No growth	
IX	2	3	7	7	1	1	No growth	
VIII	4	5	4	4	1	—	No growth	
Amphotericin B	7	8	7	8	4	4	No growth	
VII	3	2	2	2	—	—	—	—
XVII	1	1	1	1	—	—	—	—
VI	7	5	10	8	9	5	20	19
Amphotericin B	5	3	7	5	2	1	4	4

[a] Antimicrobial activity was recorded as the width (in millimeters) of the inhibition zone measured from the edge of the agar well to the edge of the inhibition zone.

Table IV—Minimum Inhibitory Concentration [a] of Cannabichromene and Cannabigerol Homologs and Isomers against Different Organisms

Compound	*B. subtilis*		*S. aureus*		*M. smegmatis*	
	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
I	0.39	0.78	1.56	1.56	12.5	25.0
III′	0.78	3.12	NT[b]	NT	25.0	25.0
VI	6.25	12.5	12.5	12.5	12.5	12.5
Streptomycin SO_4	6.25	25.0	3.12	12.5	1.56	1.56
II	3.12	3.12	3.12	3.12	3.12	6.25
VII	6.25	6.25	12.5	12.5	12.5	12.5
Streptomycin SO_4	3.12	6.25	6.25	6.25	6.25	6.25
V	1.56	1.56	0.78	3.12	3.12	3.12
Streptomycin SO_4	12.5	100	25.0	25.0	6.25	6.25
X	1.56	6.25	3.12	6.25	6.25	6.25
Streptomycin SO_4	No Readings					
XI	1.56	1.56	12.5	12.5	6.25	6.25
Streptomycin SO_4	6.25	12.5	25.0	50.0	6.25	6.25
XII	0.78	1.56	1.56	1.56	3.12	3.12
VIII	50	50	50	100	25	50
IX	25	50	50	100	50	50
Streptomycin SO_4	6.25	6.25	6.25	25	0.78	0.78

[a] Expressed in micrograms per milliliter. [b] Not tested.

Table V—Minimum Inhibitory Concentration [a] of Cannabichromene and Cannabigerol Homologs and Isomers against Different Fungi

Compound	*C. albicans*		*S. cerevisiae*		*T. mentagrophytes*		*A. niger*	
	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
I	NT[b]	NT	25.0	50.0	25.0	50.0		
Amphotericin B	NT	NT	3.12	3.12	NT	NT		
III′	50.0	100.0	NT	NT	NT	NT		
VI	50.0	50.0	25.0	25.0	25.0	25.0		
Amphotericin B	1.56	1.56	0.78	0.78	NT	NT		
II	NT	NT	6.25	12.5	6.25	6.25		
VII	12.5	25.0	NT	NT	6.25	6.25		
Amphotericin B	1.56	6.25	0.19	0.78	12.5	25.0		
V	NT	NT	12.5	12.5	50.0	50.0		
Amphotericin B	NT	NT	6.25	6.25	25.0	25.0		
X	25.0	25.0	12.5	12.5	6.25	25.0	50.0	50.0
Amphotericin B	6.25	6.25	3.12	6.25	25.0	25.0	50.0	50.0
XI	25.0	50.0	6.25	6.25	6.25	6.25	50.0	50.0
Amphotericin B	50.0	50.0	25.0	25.0	12.5	12.5	50.0	50.0
XII	12.5	25.0	6.25	6.25	25.0	12.5		
VIII	50.0	100.0	100.0	100.0	25.0	50.0		
IX	50.0	100.0	50.0	100.0	25.0	50.0		
Amphotericin B	12.5	25.0	3.12	6.25	6.25	50.0		

[a] Expressed in micrograms per milliliter. [b] Not tested.

It can be concluded from Tables III and IV that the antimicrobial activity of the normal series, in cannabichromene and cannabigerol homologs and isomers, is more pronounced than in case of the iso-series. Cannabichromene type compounds having a methyl or a pentyl group in the side chain show the highest antimicrobial activity. An intermediate type of activity is seen when R is a hydrogen. However, lengthening the side chain up to C_{15} leads to a tremendous decrease in activity. Total saturation of the two double bonds in the cannabichromene and cannabigerol type compounds having a methyl side chain in most of the cases leads to an increase in the antifungal and antibacterial activities as in the case of Compounds XII and V, respectively (Tables IV and V).

This is the first reported synthesis and spectral data of most of the compounds prepared in this investigation. In addition, the antimicrobial activities of these compounds show encouraging results. The activities of the compounds prepared in this report were compared qualitatively with those of some known cannabinoids, namely, cannabigerol, cannabidiol, cannabicyclol, Δ^8- and Δ^9-tetrahydrocannabinols and were found to be far superior in most instances (Tables II and III).

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Preparation of Hydrophilic Albumin Microspheres Using Polymeric Dispersing Agents

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Abstract □ A new method for preparing glutaraldehyde cross-linked human serum albumin microspheres has been developed. Important aspects of this method include addition of glutaraldehyde in the organic phase and use of concentrated solutions of hydrophobic polymers (polymethylmethacrylate) or hydrophilic polymers (polyoxyethylene–polyoxypropylene block copolymer) as dispersion media. Uniform, round, solid, 3–150-μm hydrophilic microspheres were readily prepared by this process. The average size of microspheres was a function of dispersion time and energy input. Surface properties were altered by chemical modification using either 2-aminoethanol or aminoacetic acid to quench residual aldehyde groups. Optical and scanning electron microscopy and electronic particle size characterization indicate that the process is versatile in producing solid microspheres in a wide size range. Albumin microspheres of this type are readily dispersed in aqueous media for injection, without the need for surfactants.

Keyphrases □ Microspheres—hydrophilic albumin, preparation using polymeric dispersing agents □ Polymeric dispersing agents—preparation of hydrophilic albumin microspheres □ Glutaraldehyde—cross-linked human serum albumin microspheres, preparation using polymeric dispersing agents

Insoluble drug carriers for prolonged and controlled delivery of therapeutic agents in biological systems recently have generated growing interest (1–3). Many different carrier systems have been studied, including synthetic liposomes, erythrocyte ghosts, permeable polymeric microcapsules, and solid microspheres (4–7). Each of these drug carriers has its own advantages and problems, and there are adequate reviews of the literature (8).

The use of albumin microspheres as drug carriers has been studied to an increasing extent (9, 10). Soluble human serum albumin in blood plasma is a natural circulatory drug carrier (11). Equilibrium binding to various drugs depends primarily on hydrophobic and electrostatic interactions (12). This type of drug binding eliminates the need for covalent attachment between drug and carrier and may facilitate drug release. Human serum albumin is also degraded *in vivo*. The stability of albumin microspheres is, therefore, a function of the degree of albumin cross-linking, porosity, and accessibility of microspheres to enzymatic and phagocytic processes in the body (13).

Current methods of albumin microsphere preparation involve either thermal denaturation at elevated temperatures (110–165°) or chemical cross-linking in vegetable oil or isooctane emulsions (14, 15). Because small amounts of surfactants are needed to disperse such microspheres in water, they appear to be somewhat hydrophobic because of the method of formation. Widder *et al.* (16) hypothesize that hydrophobicity is due to the polar regions of the albumin aligning at the oil–water interface to form a hydrophobic crust or mantle at room temperature. Since their process involves thermal denaturation for microsphere stabilization, a further increase in surface hydrophobicity may occur due to additional albumin conformational changes and surface binding of oil at elevated temperatures. Surface hydrophilicity is important, because a hy-

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