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# BIOTRANSFORMATION OF OLIVETOL BY *SYNCEPHALASTRUM RACEMOSUM*

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ABSTRACT.—A study of the biotransformation of olivetol by *Syncephalastrum racemosum* ATCC 18192 has led to the isolation of three metabolites, which were identified as 4′-hydroxyolivetol, 3-(3,5-dihydroxyphenyl)-1-propanol, and 3-(3,5-dihydroxyphenyl)-1-propanoic acid. The structures of the isolated metabolites were deduced by comparison of their spectral properties (pmr, cmr, ms) with those of olivetol. The absolute configuration of 4′-hydroxyolivetol was determined to be *R* by the Horeau partial resolution method. Biotransformation of olivetol therefore appears to occur by a subterminal oxidation process.

Microbial transformation studies have been conducted with many naturally occurring cannabinoids, namely $\Delta^8$-tetrahydrocannabinol, cannabidiol, $\Delta^9$-tetrahydrocannabinol, and cannabinol. These four cannabinoids are known to possess a variety of potentially useful pharmacological properties, such as anticonvulsant and antidepressant actions, bronchodilation, and lowering of intraocular pressure (1). A major emphasis of these studies is the production of novel and important compounds that are difficult to prepare by conventional synthetic methods (2).

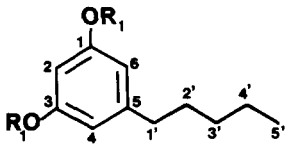
A common structural feature of most of the naturally occurring cannabinoids is the *n*-pentyl side-chain. Metabolism of this alkyl moiety is a common pathway in microorganisms (2) as well as in mammals (3). Previous studies indicated that *Syncephalastrum racemosum* ATCC 18192 was capable of partial degradation of the *n*-pentyl side-chain; highest yields of transformation were realized with cannabidiol as substrate (4). In order to characterize fully the pathways of side-chain metabolism that are typical of a given organism, a study of the biotransformation of olivetol (**1**) by *S. racemosum* has been conducted. Olivetol serves as an experimental model of the *n*-pentyl resorcinol moiety of cannabinoids.

## RESULTS AND DISCUSSION

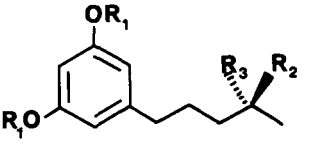
Based on initial screening experiments with a variety of fungi and bacteria, *S. racemosum* was the only organism found capable of biotransformation of olivetol in reasonable yields. A time-course study monitored by tlc revealed that 4′-hydroxyolivetol (**2**), was produced after 4-5 days of incubation, while 8-10 days were required to produce 3-(3,5-dihydroxyphenyl)-1-propanol (**3**) and 3-(3,5-dihydroxyphenyl)-1-propanoic acid (**4**). Preparative-scale fermentation was performed to isolate sufficient quantities of the metabolites for structure elucidation.

The mass spectrum of 4′-hydroxyolivetol gave a molecular ion of *m/z* 196, indicating a monohydroxylation of the parent compound. Hydroxylation occurred on the side-chain, as peaks of *m/z* 124 and 123 were observed. These ions result from benzylic cleavage between C-1′ and C-2′ with and without proton transfer, respectively, back to the aromatic ring. The same peaks (of comparable intensity) are observed in the mass spectrum of olivetol. The cmr spectrum also indicated that hydroxylation had occurred on the side-chain. Resonances for the aromatic carbons (Table 1) are quite similar for both olivetol and 4′-hydroxyolivetol. However, a doublet at 67.68 ppm in the off-resonance decoupled spectrum is observed that is not present in the spectrum of olivetol. The side-chain $^{13}$C-resonances for olivetol were assigned based on reported values for other cannabinoids (5).

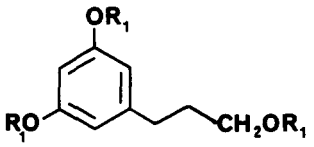
The exact position of hydroxylation was determined as C-4′ from the pmr spectrum. A doublet signal at δ1.10 (C-5′-$CH_3$) was present, while the triplet at δ0.86 for the C-5′-$CH_3$ of olivetol was absent. This was further verified from an analysis of the mass spectrum of the trimethylsilyl (TMSi) ether (**2a**) of 4′-hydroxyolivetol. A peak of *m/z* 117 is seen. This ion is prominent in the mass spectra of a wide variety of compounds that are hydroxylated in the penultimate (ω-1) position of the side-chain (6). The ion of *m/z* 117 is presumably formed by cleavage in the substituted alkyl side-chain, resulting in the formation of the fragment $[CH_3CH\text{-}OTMSi]^+$ (7).



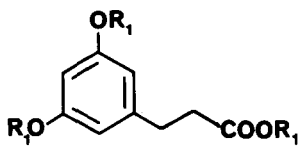
**1** $R_1$=H
**1a** $R_1$=Si($CH_3$)$_3$



**2** $R_1$=$R_2$=H; $R_3$=OH
**2a** $R_1$=Si($CH_3$)$_3$; $R_2$=H; $R_3$=OSi($CH_3$)$_3$
**2b** $R_1$=$CH_3$; $R_2$=H; $R_3$=OH
**2c** $R_1$=$CH_3$; $R_2$=H; $R_3$=$C_2H_5CH(C_6H_5)COO$
**2d** $R_1$=$CH_3$; $R_2$=$R_3$=O
**2e** $R_1$=$CH_3$; $R_2$=H; $R_3$=$C_6H_5C(OCH_3)(CF_3)COO$ (MTPA ester of 2b)
**2f** $R_1$=$CH_3$; $R_2$=H; $R_3$=$C_6H_5C(OCH_3)(CF_3)COO$ (MTPA ester of racemic 2b)



**3** $R_1$=H
**3a** $R_1$=Si($CH_3$)$_3$



**4** $R_1$=H
**4a** $R_1$=Si($CH_3$)$_3$

Hydroxylation of olivetol at the 4′-position introduces a chiral center into a previously achiral molecule. 4′-Hydroxyolivetol exhibits a small negative optical rotation. Application of the Horeau method for establishment of configuration of secondary alcohols (8) determined the absolute stereochemistry. Esterification of the dimethyl ether derivative (**2b**) with optically inactive α-phenylbutyric anhydride resulted in a recovery of the (+) α-phenylbutyric acid in an optical yield of 15.6%. This requires the *R* configuration for the arrangement of substituents at C-4′.

The enantiomeric composition of 4′-hydroxyolivetol was determined via Mosher's method (9). Analysis of the 500 MHz pmr spectrum of the spectrum of the α-methoxy-α-trifluoromethylphenyl acetate (MTPA) (**2e**) revealed two sets of peaks for the MTPA-$OCH_3$ moiety at δ 3.56 and 3.53. These were compared to the resonances for the same group in the MTPA ester **2f** of racemic 4′-hydroxy-dimethylolivetol (prepared from **2b** via Jones' oxidation and $NaBH_4$ reduction, followed by derivatization) and found to be identical (Figure 1). Integration of the -$OCH_3$ resonances revealed a 1:1 ratio for MTPA ester **2f** but a 10:1 ratio for MTPA ester **2e**, thus indicating that an 82% enantiomeric excess of the *R* isomer of 4′-hydroxyolivetol is made by *S. racemosum*.

The mass spectrum of 3-(3,5-dihydroxyphenyl)-1-propanol gave a molecular ion of *m/z* 168, consistent with the loss of a -$CH_2CH_3$ group and introduction of an oxygen

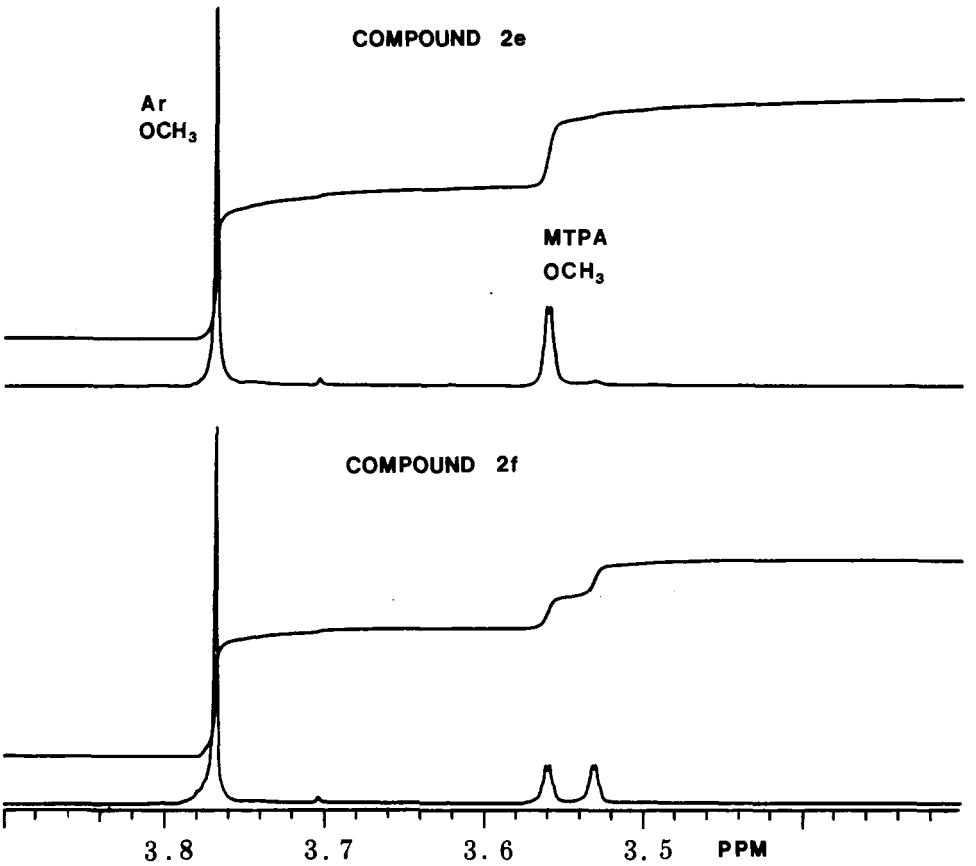



FIGURE 1. 500 MHz pmr data for MTPA esters **2e** and **2f**.

atom. The base peak of *m/z* 124, resulting from benzylic cleavage, indicated that the oxygenation occurred in the side-chain. The cmr (Table 1) revealed no changes for the aromatic carbons, but a loss of two carbons from the *n*-pentyl moiety. The hydroxylated carbon atom exhibits a peak at 61.95 ppm. The pmr spectrum showed triplets at δ 3.56 and 2.52 for the C-3′ and C-1′ methylenes, respectively. The downfield position of the resonance for C-3′ indicates hydroxylation of this carbon.

TABLE 1. Cmr Spectral Data for Olivetol and Metabolites

| *Carbon* | **1** | **2** | **3**[a] | **2b** |
|---|---|---|---|---|
| 1,3 | 156.18 (s) | 158.98 (s) | 159.33 | 160.97 (s) |
| 2 | 100.69 (d) | 100.93 (d) | 101.05 | 97.96 (d) |
| 4,6 | 108.66 (d) | 107.65 (d) | 107.74 | 106.73 (d) |
| 5 | 146.66 (s) | 145.65 (s) | 145.47 | 144.97 (d) |
| 1′ | 35.87 (t)[b] | 36.46 (t)[c] | 35.21[c] | 36.31[c,d] |
| 2′ | 30.63 (t) | 27.96 (t) | 32.89[c] | 27.42[d] |
| 3′ | 31.58 (t) | 39.49 (t)[c] | 61.95 | 39.02[c,d] |
| 4′ | 22.55 (t) | 67.68 (d) | — | 68.10 (d) |
| 5′ | 13.98 (q) | 23.67 (q) | — | 23.67 (q) |
| $-OCH_3$ | — | — | — | 55.37 (q) |

[a]Due to insufficient quantities of material, off-resonance decoupled spectrum was not obtained.
[b]For positions of cmr resonances for side-chain carbons of other cannabinoids, see Archer *et al.* (5).
[c]Assignments in any vertical column may be reversed.
[d]Multiplicity could not be determined from off-resonance decoupled spectrum.

The mass spectrum of 3-(3,5-dihydroxyphenyl)-1-propanoic acid gave a molecular ion of *m/z* 182. Benzylic cleavage, giving the peak at *m/z* 123, indicates that the *n*-pentyl moiety has been modified. Typical of carboxylic acids, a loss of COOH gives rise to the base peak of *m/z* 137. The ir spectrum confirms the presence of a carboxylic acid, as a broad OH stretching vibration at 3700-2800 $cm^{-1}$ (COOH superimposed on phenolic OH) and a carbonyl absorption band at 1710 $cm^{-1}$ are observed. The pmr spectrum exhibits a multiplet for the C-1′ and C-2′ methylenes at 2.64.

Based on the above evidence, it is suggested that the pentyl side-chain of olivetol is metabolized by *S. racemosum* via a subterminal oxidation pathway, as shown in Figure 2. Hydroxylation occurs at C-4′ to give 4′-hydroxyolivetol, which may be followed by oxidation to the ketone. A microbial Baeyer-Villiger oxidation could give rise to the proposed acetate intermediate which is cleaved to give the primary alcohol product **3**. Further oxidation of the alcohol may form the carboxylic acid metabolite **4**. It seems logical that metabolite **4** could serve as a substrate for β-oxidation. However, no products of further metabolism could be isolated, although trace quantities of other polar metabolites were seen by tlc. Subterminal oxidation is a common pathway for the microbial degradation of alkanes. For example, *Pseudomonas aeruginosa* metabolizes tridecane in this fashion, while a *Penicillium* species degrades tetradecane using this pathway (10).

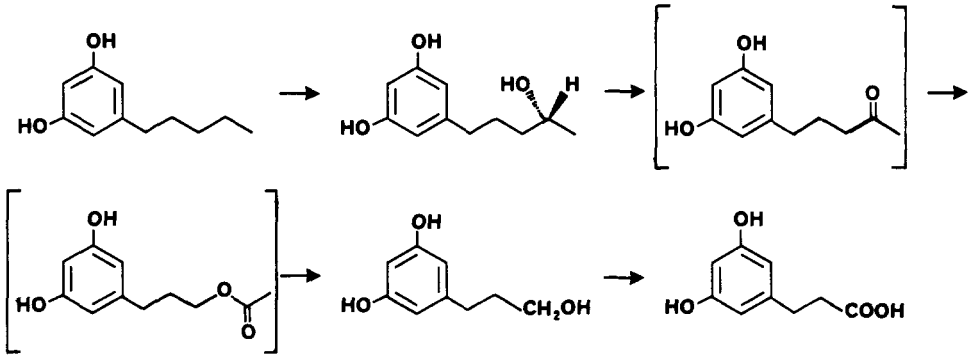



FIGURE 2. Sub-terminal Oxidation of Olivetol by *Syncephalastrum racemosum* ATCC 18192

The results with olivetol are analogous to those observed with cannabidiol (2,4), although better biotransformation yields are obtained with olivetol. *S. racemosum* produces 4′-hydroxyolivetol in 30% yield, while 4′-hydroxycannabidiol is made by this organism in 5% yield. Knowledge of this pathway has potential utility for the production of specific side-chain metabolites of cannabinoids for pharmacological testing. Determination of the absolute stereochemistry of 4′-hydroxyolivetol should contribute to the understanding of stereochemical aspects of side-chain hydroxylation, in both microbial and mammalian systems.

## EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were determined with a Beckman model 4230 Infrared Spectrometer. Uv spectra were determined in 95% EtOH with a Beckman model 5260 Ultraviolet and Visible Spectrometer. Pmr spectra were determined in the stated solvents with TMS as the internal standard on a Bruker HX90 (90 MHz) instrument equipped for pulse mode with Fourier transform analysis or on a GE/Nicolet NT-500 spectrometer. Cmr spectra were obtained with a Bruker WP80 instrument at 20.1 MHz. Chemical shifts are reported in ppm (δ) and coupling constants in Hz. Optical rotations were measured with a Perkin-Elmer 241 Photoelectric Polarimeter. Melting points were determined in open-end capillaries in a Thomas-Hoover Unimelt apparatus and are uncorrected. Electron-impact mass spectra were determined with a Dupont 21-491 mass spectrometer (70 eV). Trimethylsilyl-derivatized compounds were analyzed with a Finnegan 4021 Automated Gas Chromatograph/EI-CI System.

CULTURE METHODS.—*S. racemosum* ATCC 18192 was obtained from the American Type Culture Collection. The fungus was maintained on Mycophil (BBL) agar slants stored at 4°. Periodic transfer (every 2-3 months) preserved the culture.

Shaken cultures for biotransformation experiments were generated by a two-stage fermentation procedure (11) in a medium consisting of (per liter of $H_2O$): yeast extract, 3 g; malt extract, 3 g; peptone, 5 g; dextrose, 10 g. Stage I cultures were initiated by pipetting an aqueous spore suspension prepared from 7-day-old slant cultures into 100 ml of medium in a 500-ml Erlenmeyer flask. The Stage I cultures were incubated for 3 days (250 rpm, 25°), and then 10 ml of culture was withdrawn to inoculate each Stage II culture. The Stage II cultures were used for olivetol biotransformation studies.

ANALYTICAL METHODS.—The time course of olivetol biotransformation was monitored using whole cultures or aliquots of cultures by tlc on 0.25 mm silica gel 60 F-254 plates (E. Merck), which were developed in the specified solvent system. Chromatograms were visualized by fluorescence quenching under 254 nm uv light and by spraying with a 0.5% aqueous solution of Fast Blue BB salt (Aldrich Chemical Co.). Preliminary identification of metabolites was performed by gc/ms of TMSi-derivatized compounds. TMSi derivatives were prepared by addition of 15 μl pyridine and 25 μl (bis-trimethyl)-trifluoroacetamide (BSTFA, Pierce Chemical) to 1-2 mg of crude culture extract. The mixture was heated at 120° for 30 min, and excess reagent was removed under $N_2$. The residue was redissolved in 20 μl hexane and submitted for gc/ms analysis. The gc was equipped with a 3% OV-17 column (glass, 6 ft×2 mm). The injection volume was 1 μl, and samples were run isothermally at 220° (injector 250°).

OLIVETOL (**1**).—Olivetol was purchased from Aldrich Chemical Company and exhibited the following physical properties: mp 42-44°; uv λ max (EtOH) (log ε) 280 (3.26), 274 (3.26), 204 (4.60); ir ν max (melt) 3320, 2920, 2850, 1600, and 1140 $cm^{-1}$; pmr ($CDCl_3$, 90 MHz), δ 0.86 (3H, t, $J$=6, 5′-$CH_3$), 1.22-1.68 (6H, m, 2′-, 3′-, 4′-$CH_2$), 2.43 (2H, t, $J$=7, 1′-$CH_2$), 5.62 (2H, broad s, exchanges with $D_2O$, OH), 6.17 (1H, t, $J$=2, 2-ArH), 6.26 (2H, d, $J$=2, 4-, 6-ArH); cmr ($CDCl_3$), see Table 1; ms *m/z* (% relative abundance) 180 (46), 138 (18.2), 137 (22.8), 125 (16.2), 124 (100), 123 (52.6); ms (TMSi ether **1a**), *m/z* (% relative abundance) 324 (16.2), 282 (12.2), 269 (23.3), 268 (100), 73 (97).

TRANSFORMATION OF OLIVETOL BY *SYNCEPHALASTRUM RACEMOSUM* ATCC 18192.—Olivetol (**1**) was dissolved in absolute EtOH (840 mg/16.8 ml) and distributed evenly among 42 48-hour-old Stage-II cultures. The cultures were incubated with shaking (250 rpm, 25°). Tlc analysis 7-8 days after substrate addition, using $CHCl_3$-MeOH (4:1), indicated that three metabolites, **2** (Rf 0.43), **3** (Rf 0.37), and **4** (Rf 0.19), were present, in addition to unchanged olivetol, **1** (Rf 0.58). The cultures were harvested by filtration. The filtrate was acidified to pH 3 with concentrated HCl and exhaustively extracted with $CHCl_3$ first and then with EtOAc. The $CHCl_3$ extract contained most of the unchanged starting material, while the EtOAc extract contained most of the metabolites. The EtOAc extract was concentrated to a brown non-drying oil (1.54 g).

The concentrated EtOAc extract was applied to a silica gel PF 254 (E. Merck) column (250 g, 65 cm×3.5 cm) and eluted with $CHCl_3$-MeOH (9:1); 200 fractions of 10 ml each were collected. Metabolite **2** was eluted in fractions 43-50 (156 mg), while mixtures of **2** and **3** were obtained in fractions 51-65 (217 mg). Fractions 66-111 contained mainly **3** and **4** (100 mg).

The mixture of **2** and **3** (fractions 51-65) was applied to a silica gel PF254 column (60 g, 48×1.75 cm) and eluted with $CHCl_3$-MeOH-$H_2O$ (120:30:8, lower layer). A total of 100 fractions of 3.5 ml were collected. Fractions 38-53 from this column contained **2** (117 mg), while fractions 63-73 contained **3** (39 mg).

The mixture of **3** and **4** (fractions 66-111) was separated with a Chromatotron (Harrison Research Inc.), a centrifugally accelerated radial thin layer chromatograph, using a 2-mm coating of silica gel 60 (PF254, E. Merck), eluting with $CHCl_3$-MeOH-$H_2O$ (120:50:15, lower layer). Approximately 100 fractions of 2.5 ml were collected. Metabolite **4** (15 mg) eluted with an EtOAc rinse of the Chromatotron plate. No traces of this compound could be found in the first 100 fractions.

CHARACTERIZATION OF 4′-HYDROXYOLIVETOL [1-(3,5-DIHYDROXYPHENYL)-4*R*-PENTANOL] (**2**).—Pure fractions of metabolite **2** were combined to yield 273 mg of a brown oil (29.8% yield). A small sample was crystallized from MeOH, mp 91.5°-93°. The compound exhibited the following spectral properties: uv λ max (EtOH) (log ε) 281 (3.14), 274 (3.17), 202 (4.56); ir ν max (neat) 3300, 2960, 2860, 1600, and 1150 $cm^{-1}$; pmr ($Me_2CO$-$d_6$, 90 MHz), δ 1.10 (3H, d, $J$=6, 5′-$CH_3$), 1.31-1.84 (4H, m, 2′-, 3′-$CH_2$), 2.46 (2H, t, $J$=7, 1′-$CH_2$), 3.09 (2H, broad s, exchanges with $D_2O$, OH), 3.63-3.82 (1H, m, 4′-CH), 6.18 (2H, s, 4-, 6-ArH); cmr ($Me_2CO$-$d_6$), see Table 1; ms *m/z* (% relative abundance) 196 (31.4), 178 (19.5), 136 (86.7), 124 (89.7), 123 (65.7), 111 (100); ms (TMSi ether **2a**), *m/z* (% relative abundance) 412 (10), 280 (44.6), 268 (31.9), 117 (31), 73 (100); $[\alpha]^{24}D$ −8.8° (c 1.6, MeOH).

CHARACTERIZATION OF 3-(3,5-DIHYDROXYPHENYL)-1-PROPANOL (**3**).—The metabolite **3** was further purified by preparative tlc in $CHCl_3$-MeOH-$H_2O$ (120:30:8, lower layer) and crystallized from

EtOAc to yield 22 mg of fibrous white crystals (2.8% yield): mp 110°-112°; uv λ max (EtOH) (log ε) 281 (3.13), 275 (3.16), 202 (4.50); ir ν max (neat, non-crystalline sample) 3300, 2940, 1600, and 1140 $cm^{-1}$; pmr ($Me_2CO$-$d_6$, 90 MHz), δ 1.61-1.85 (2H, m, 2'-$CH_2$), 2.52 (2H, t, $J$=8, 1'-$CH_2$), 3.56 (2H, t, $J$=6, 3'-$CH_2$), 6.19 (2H, s, 4-, 6-ArH), 8.00 (1H, broad s, exchanges with $D_2O$, OH); cmr ($Me_2CO$-$d_6$), see Table 1; ms *m/z* (% relative abundance) 168 (21.6), 124 (100), 123 (28.8); ms (TMSi ether **3a**), *m/z* (% relative abundance) 384 (2), 269 (24.1), 268 (100), 73 (49.8).

CHARACTERIZATION OF 3-(3,5-DIHYDROXYPHENYL)-1-PROPANOIC ACID (**4**).—The metabolite **4** crystallized as cream-colored plates (15 mg, 1.8% yield); mp 117°-119°; lit. 125° (12); uv λ max (EtOH) (log ε) 281 (3.32), 274 (3.34), 202 (4.63); ir ν max (neat, non-crystalline sample) 3280, 1705, 1610, and 1160 $cm^{-1}$; pmr ($Me_2CO$-$d_6$, 90 MHz), δ 2.42-2.86 (4H, m, 1'-, 2'-$CH_2$), 6.18-6.22 (3H, m, 2-, 4-, 6-ArH), 6.5 (2H, broad s, exchanges with $D_2O$, OH); ms *m/z* (% relative abundance) 182 (79.8), 137 (100), 123 (26.2); ms (TMSi ether **4a**), *m/z* (% relative abundance) 398 (1.9), 281 (11.4), 117 (20.3), 73 (100).

PREPARATION OF 4'-HYDROXY-DIMETHYLOLIVETOL (**2b**).—Compound **2** (107 mg, 0.546 mmole) was dissolved in 2 ml MeOH. To this was added an excess of freshly distilled ethereal $CH_2N_2$ (generated from DIAZALD, Aldrich Chemical Co.). The flask was stoppered and allowed to stand at ambient temperature for 24 h. Removal of solvent and preparative tlc in $CHCl_3$-EtOH (92:8) yielded 77 mg of **2b** as a yellow oil with the following properties: Rf 0.48 with $CHCl_3$-EtOH (92:8); uv λ max (EtOH) (log ε) 280 (3.29), 272 (3.30), 203 (4.72); ir ν max ($CHCl_3$) 3600, 3460, 2960, 2860, 1600, 1195, 1160, and 1060 $cm^{-1}$; pmr ($CDCl_3$, 90 MHz) δ 1.18 (3H, d, $J$=6, 5'-$CH_3$), 1.32-1.82 (4H, m, 2'-, 3'-$CH_2$), 2.57 (2H, t, $J$=7, 1'-$CH_2$), 3.70-3.90 (7H, -$OCH_3$ s+4'-CH m), 6.30-6.33 (3H, m, 2-, 4-, 6-ArH); cmr ($CDCl_3$), see Table 1; ms *m/z* (% relative abundance) 224 (55.4), 191 (25.1), 165 (40.8), 164 (41.1), 152 (68.7), 151 (50), 139 (100); $[\alpha]^{24}D$ −9.65° (c 2.75, MeOH).

PREPARATION OF α-PHENYLBUTYRATE ESTER (**2c**).—Ten milligrams of **2b** (0.045 mmole) and 40.1 mg of α-phenylbutyric anhydride (0.129 mmole) were stirred in 2.5 ml of dry pyridine for 43 h at ambient temperature; 1 ml $H_2O$ was added and stirring was continued for 3 h. The reaction mixture was further diluted with 8 ml $H_2O$ and extracted with $Et_2O$. The $Et_2O$ layer was successively extracted with $H_2O$ (3×10 ml), 5% $NaHCO_3$ (3×10 ml), and $H_2O$ (3×10 ml). All the aqueous layers were combined, acidified to pH 2 with 1 *N* $H_2SO_4$, and extracted with $CHCl_3$. The $H_2O$-washed $CHCl_3$ layer gave 29.5 mg of α-phenylbutyric acid as white crystals: mp 41°-42°; lit. 41° (8); $[\alpha]^{25}D$ +1.89° (c 2.65, $C_6H_6$). The $Et_2O$ fraction still contained some starting material by tlc and was therefore purified by preparative tlc in hexane-EtOAc (2:3) to yield 9.7 mg of **2c**. This corresponds to an isolated yield of the ester of 61% and therefore an optical yield of α-phenylbutyric acid of 15.6%. Compound **2c** exhibited the following properties: Rf 0.63 with hexane-EtOAc (2:3); uv λ max (EtOH) (log ε) 280 (3.33), 272 (3.34), 204 (4.80); ir ν max ($CHCl_3$) 3005, 2960, 2870, 1730, 1600, 1495, 1160, and 1070 $cm^{-1}$; ms *m/z* (% relative abundance) 370 (14.6), 223 (14.5), 207 (27.7), 206 (38.2), 191 (41.7), 164 (76), 152 (79.2), 151 (78.2), 119 (34.9), 91 (100), 77 (17.4); $[\alpha]^{25}D$ −18° (c 0.55, MeOH).

PREPARATION OF 4'-OXO-DIMETHYL OLIVETOL (**2d**).—Compound **2b**, 27.9 mg (0.125 mmole), was dissolved in 2 ml $Me_2CO$ and cooled to 0°; 0.25 ml of Jones' reagent (13) was added and the reaction mixture stirred for 5 min. The reaction mixture was then poured into 50 ml $H_2O$ and extracted twice with 50 ml $CH_2Cl_2$. The $CH_2Cl_2$ extract was dried to yield a yellow oil, which was purified by preparative tlc in hexane-EtOAc (2:3) to yield 16.5 mg of **2d**, which exhibited the following properties: Rf 0.49 with hexane-EtOAc (2:3); uv λ max (EtOH) (log ε) 279 (3.45), 272 (3.48), 203 (4.72); ir ν max ($CHCl_3$) 1710, 1600, and 1160 $cm^{-1}$; pmr ($CDCl_3$, 90 MHz) δ 1.80-2.04 (2H, m, 2'-$CH_2$), 2.12 (3H, s, 5'-$CH_3$), 2.44 (2H, t, $J$=7, 3'-$CH_2$), 2.57 (2H, t, $J$=7, 1'-$CH_2$), 3.77 (6H, s, -$OCH_3$), 6.32 (3H, s, 2-, 4-, 6-ArH); ms *m/z* (% relative abundance) 222 (67.3), 165 (43.6), 164 (100), 152 (59.8), 121 (11.1).

PREPARATION OF RACEMIC **2b**.—Compound **2d**, 15 mg (0.067 mmole), was dissolved in 2 ml MeOH and cooled to 0° with constant stirring. To this was added 50 mg $NaBH_4$ (1.32 mmole) and stirring was continued for 15 min. The reaction mixture was extracted twice with 10 ml EtOAc and the EtOAc extract was dried to yield 13.6 mg of a yellow oil with the following properties: Rf 0.38 with hexane-EtOAc (2:3); pmr ($CDCl_3$, 90 MHz), identical to that of **2b**; $[\alpha]^{23}D$ −0.3° (c 0.4, $CHCl_3$). Lack of significant optical rotation indicated the compound was racemic.

PREPARATION OF MTPA ESTER **2e**.—Compound **2b**, 26 mg (0.116 mmole), and (−) α-methoxy-α-trifluoromethylphenylacetyl chloride, 69.5 mg (0.275 mmole), were dissolved in 1 ml dry pyridine and stirred at ambient temperature for 20 h. The reaction was judged to be complete by tlc. To hydrolyze excess acid chloride, 1 ml of $H_2O$ was added and stirring continued for 1 h. The reaction mixture was then extracted with 20 ml $CHCl_3$ and the $CHCl_3$ layer was then successively extracted with $H_2O$ (3×10 ml), 5% $NaHCO_3$ (3×10 ml), and $H_2O$ (3×10 ml). The $CHCl_3$ layer was evaporated to give 40 mg of a yellow oil which was used for 500 MHz pmr studies and which exhibited the following properties: Rf 0.70 with

hexane-EtOAc (2:3); uv λ max (EtOH) (log ε) 280 (3.21), 274 (3.23), 204 (4.66); ir ν max ($CHCl_3$) 3005, 1745, 1600, 1220, and 770 $cm^{-1}$; ms *m/z* (% relative abundance) 440 (50.8), 207 (45.6), 206 (28.7), 191 (35.9), 189 (62.5), 152 (69.3), 151 (100), 91 (12.6), 77 (18.8); $[\alpha]^{24}D$ −42.3° (c 0.4, $CHCl_3$).

PREPARATION OF MTPA ESTER **2f**.—Racemic **2b**, 10 mg (0.045 mmole), and (−) α-methoxy-α-trifluoromethylphenylacetyl chloride, 62 mg (0.246 mmole), were dissolved in 1 ml dry pyridine and stirred at ambient temperature for 23.5 h (reaction complete by tlc), followed by addition of 0.5 ml $H_2O$ and continued stirring for 1 h. The reaction mixture was evaporated to dryness and reconstituted in 20 ml $CHCl_3$, and successively extracted with $H_2O$ (3×15 ml), 5% $NaHCO_3$ (3×15 ml), and $H_2O$ (3×15 ml). The $CHCl_3$ layer was evaporated to yield 19 mg of a yellow oil which was used for 500 MHz pmr studies and which exhibited the following properties: Rf 0.70 with hexane-EtOAc (2:3); uv λ max (EtOH) (log ε) 280 (3.22), 274 (3.25), 204 (4.61); ir ν max ($CHCl_3$) 3000, 1740, 1610, 1600, 1210, and 750 $cm^{-1}$; ms *m/z* (% relative abundance) 440 (65.4), 207 (38.7), 206 (32.2), 191 (40.1), 189 (62.9), 152 (72.5), 151 (100), 91 (13.9), 77 (18.3); $[\alpha]^{24}D$ −24.5° (c 0.5, $CHCl_3$).

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