and their effectiveness as inhibitors are observed, a case could be made for smaller molecular volumes due to hydrophobic interaction between the naphthyl ring and the amino acid side chain. A similar consideration might apply to dansyl-L-proline, an amino acid derivative of more rigid structure.

If one exempts the derivatives of phenylalanine and the leucines and the dansyl derivative of proline from the rest of the compounds, a correlation coefficient for K_i and lipid solubility is found to be 0.739.

One might consider the tritium isotope effect from the [*N-methyl*-^{3}H]dansylamide and dansyl acid and to what degree this could potentially effect the observed rates of N-demethylation. The rate of N-demethylation of the nonlabeled material is not expected to be significantly higher than that measured since, first, we do not measure small, initial, but rather significant overall conversions; second, Thompson et al.[18] have found that colorimetric and radioactive assays of [*N-methyl*-^{3}H]ethylmorphine N-demethylation yield identical quantitative values. In addition, Elison et al.[21,22] have compared the rates of demethylation of morphine (R-NH-CH_3) and trideuterio-*N*-methylmorphine (R-NH-CD_3) by rat liver microsomes. The rate of demethylation of the deuterated substrate was only slightly less than that of the hydrogenated compound. Another parameter which might affect the overall drug metabolic rates is that amino acids are actively transported, and it is possible that their dansylated derivatives are also substrates for these transport systems. These agents could also affect the drug-metabolizing system at a number of other points such as NADPH synthesis or respiration; however, the competitive kinetics argue against such alternative mechanisms.

The isolated liver cells are definitely a complicated system and to study the dependence of drug metabolism on lipid solubility, similar experiments will have to be carried out with liver microsomes. The comparison of these two sets of data will then allow the evaluation of the question of whether the anomalies which we have observed are inherent to the P-450 system or are introduced by the multicompartmentation of the intact cell. It should be pointed out that liver cells constitute a higher level of biological organization, so that information collected in liver cell experiments should be closer to the in vivo situation than experiments performed with microsomes.

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Notes

Hashish.[1] Unsaturated Side-Chain Analogues of Δ^8-Tetrahydrocannabinol with Potent Biological Activity

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Two Δ^8-THC derivatives, **4a** and **4b**, with functionalized side chains were synthesized. Treatment of (+)-*trans*-*p*-mentha-2,8-dien-1-ol with the resorcinal **2b** followed by removal of the dithiol group with HgO-BF_3·Et_2O gave the aldehyde **3b**. A Wittig reaction of dimethyl (2-oxoheptyl)phosphate with **3b** furnished **4a**, which was reduced to **4b**. Compounds **4a** and **4b** showed potent cannabinoid-like activity in mice.

The main active constituent of marihuana is Δ^9-tetrahydrocannabinol (THC) which causes CNS depression and ataxia in laboratory animals. The most prominent effects are decreased locomotor activity, increased sensitivity to stimuli, such as sound and touch, general depression, and, at high doses, static and dynamic ataxia.[2]

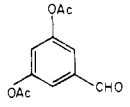
In natural THC's, i.e., Δ^8- and Δ^9-THC's, the effect of side-chain modification in the aromatic ring has not been studied as extensively as in the synthetic $\Delta^{6a,10a}$-THC's. However, the limited examples which have been reported[3]

seem to parallel the structure activity of the synthetic $\Delta^{6a,10a}$-THC's. Thus the biological activity, as measured by ataxia in the dog or sensitivity to touch in the mouse, decreases when the side chain is changed from 1,2-dimethylheptyl to n-C_5H_{11} to n-propyl in both the Δ^8 and Δ^9 series. In the monkey,[3] however, the minimum effective dose (MED) for general CNS depression does not change noticeably in the 1,2-dimethylheptyl analogue compared to Δ^9-THC. The only striking difference seen is the prolongation of activity in the former. It has also been shown that the introduction of a hydroxyl group at C_1 in the side chain[3,4] of Δ^8-THC decreases activity since the compound was inactive up to 5 mg/kg in the monkey whereas Δ^8-THC was active at 0.1 mg/kg. As a part of our ongoing program of structure–activity modification of natural THC's, we prepared the Δ^8-THC derivatives **4a** and **4b** with functionalized side chains. In this paper, we report their synthesis and show that this side-chain modification results in the retention of potent biological activity.

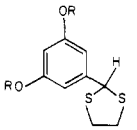
3,5-Diacetoxybenzaldehyde (1)[5] was allowed to react with 1,2-ethanedithiol in the presence of dry HCl to give **2a** which was hydrolyzed with KOH to the resorcinol **2b**. Condensation of **2b** with (+)-*trans*-*p*-mentha-2,8-dien-1-ol and a catalytic amount of *p*-toluenesulfonic acid (*p*-TSA) gave[6] the Δ^8-THC derivative **3a**. The protecting dithiol group was removed using the HgO–BF_3–Et_2O procedure[4] to form the aldehyde **3b**. A Wittig reaction with dimethyl (2-oxoheptyl)phosphonate and **3b** furnished **4a** which was reduced with $LiAlH_4$ to the alcohol **4b**.

Compound **4a** was found to be much more active than Δ^9-THC when tested in mice for ataxia and increased reactivity to sensory stimuli described by us earlier.[7] It showed a MED of <0.1 mg/kg (iv) whereas Δ^9- or Δ^8-THC's have a MED of 0.5 mg/kg.[7] Similarly, it was more active than Δ^9-THC in the mouse fighting test[8] showing an 80% decrease at 10 mg/kg po and a 96% decrease at 20 mg/kg po. The percent decrease in fighting behavior was found to be the same after 30 and 90 min at both doses. In the same test Δ^9- or Δ^8-THC's show a 50–68% decrease at 10 mg/kg.

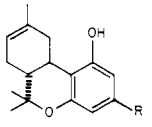
The alcohol **4b** was less active than **4a** in both the tests. It had a MED of 1.0 mg/kg iv in mice and produced a 33% decrease in the mouse fighting test at 20 mg/kg po. Both the compounds **4a** and **4b** showed little or no activity in the mouse Dopa test[8] and were inactive (up to 40 mg/kg po) in the mouse hot-plate test[8] for antinociceptive activity.

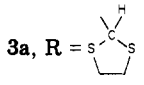


1

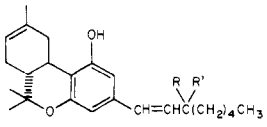


2a, R = Ac
b, R = H





3a, R = -C(H)(S)(S)
b, R = -CHO



4a, R + R' = O
b, R = H; R' = OH

Experimental Section

Melting points are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus. Elemental analyses were carried out by Spang Microanalytical Laboratories, Ann Arbor, Mich. NMR spectra were determined on a Varian T-60 instrument. Ir and NMR spectra of all compounds were consistent with the assigned structures. For GLC analysis a Varian Model 1440 instrument was used (column packing, 2% OV-17).

5-(1,3-Dithiolan-2-yl)resorcinol (2b). Dry hydrogen chloride was bubbled into a solution of 50.0 g (0.23 mol) of 3,5-diacetoxybenzaldehyde (1) and 21.4 g (0.23 mol) of 1,2-ethanedithiol in 25 ml of ether until the solution became warm. After allowing the solution to stand at ambient temperature for 0.75 h, the solvent was removed in vacuo and excess methanol was added. A solid (mp 94–96°) was separated by filtration. It was resuspended in 100 ml of methanol and 21.7 g (0.23 mol) of KOH pellets was added. On stirring the mixture at ambient temperature for 0.5 h, the mixture became homogeneous. The solvent was removed in vacuo and the residue after acidification with 6 N HCl was extracted with ethyl acetate. The extract was washed with water until neutral, dried, and evaporated in vacuo to leave **2b** as a gum, 41.3 g (84%), which was used without further purification in the subsequent reaction.

3-Formyl-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (3b). To a solution of 41.3 g (0.193 mol) of **2b** in 500 ml of benzene was added 35.3 g (0.23 mol) of (+)-*trans*-*p*-mentha-2,8-dien-1-ol and 6.8 g (0.036 mol) of *p*-toluenesulfonic acid monohydrate. An additional 250 ml of benzene was added and the solution was refluxed using a Dean-Stark trap for 0.7 h. After cooling, the mixture was washed with water, dried, and evaporated to leave 65.2 g of a gum. Purification by column chromatography on Florisil using benzene as the eluent gave 36.4 g (54%) of 3-(1,3-dithiolan-2-yl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (**3a**).

To a slurry of 28.16 g (0.13 mol) of mercuric oxide (red) in 200 ml of THF was added 43.5 ml of water and 10.9 ml (0.09 mol) of BF_3·Et_2O. With vigorous stirring a solution of 17.5 g (0.05 mol) of **3a** in 100 ml of THF was added dropwise. The color changed from bright red to a brownish red. After stirring at ambient temperature for 1 h, the mixture was filtered and to the filtrate excess ether was added. The solution was washed consecutively with water, 5% $NaHCO_3$, and again with water, dried, and evaporated to leave 12.1 g of a gum. It was purified by chromatography on 500 g of Florisil and eluted with $CHCl_3$ to yield 7.6 g (56%) of **3b**: GLC showed it to be >90% pure; NMR δ ($CDCl_3$) 1.10 (s, 3 H, α-CH_3 at C_6), 1.41 (s, 3 H, β-CH_3 at C_6), 1.71 (br s, 3 H, olefinic CH_3), 2.83 (ddd, 1 H, H_{10a}), 3.33 (dd, 1 H, $H_{10\alpha}$), 5.46 (br s, 1 H, vinylic), 6.84 (br s, 1 H, D_2O exchangeable), 6.92 (d, 1 H, aromatic H_4), 7.02 (d, 1 H, aromatic H_2), 9.81 (1 H, CHO); ir (neat) 3370, 1680, 1582 cm^{-1}.

3-(3-Oxo-1-octenyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (4a). A mixture of 7.55 g (0.028 mol) of **3b** and 6.16 g (0.028 mol) of dimethyl (2-oxoheptyl)phosphonate was added dropwise to a slurry of 1.34 g (0.056 mol) of NaH (57% oil dispersion) in 1,2-dimethoxyethane. After addition was complete, the mixture was stirred at 85° for 1 h. After cooling, the excess NaH was decomposed by the slow addition of 1:1 ether–methanol mixture followed by excess water. The aqueous phase was extracted three times with ether and the ethereal solution was dried and concentrated in vacuo to give 8.1 g of a gum. Purification by chromatography on 300 g of Florisil and elution with 1:4 ethyl acetate–hexane furnished 5.6 g (56%) of **4a** as a tan solid: mp 119–122°; NMR δ ($CDCl_3$) 0.88 (t, 3 H, ω-CH_3), 1.07 (s, 3 H, α-CH_3 at C_6), 1.37 (s, 3 H, β-CH_3 at C_6), 1.68 (br s, 1 H, olefinic CH_3), 2.64 (t, 2 H, -$COCH_2$), 3.38 (br d, 1 H, $H_{10\alpha}$), 5.45 (br s, 1 H, vinylic), 6.64, 7.48 (2 d, 2 H, J = 16 Hz, vinylic side chain), 6.73, 6.83 (2 d, 2 H, aromatic); ir (neat) 3360, 1640 (br), 1610, 1573 cm^{-1}. Anal. ($C_{24}H_{32}O_2$) C, H.

3-(3-Hydroxy-1-octenyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6*H*-dibenzo[*b*,*d*]pyran-1-ol (4b). A solution of 4.6 g (0.013 mol) of **4a** in ether was added dropwise to a slurry of 0.34 g (0.009 mol) of $LiAlH_4$ in ether. After addition was complete, the mixture was stirred at room temperature for 1 h and decomposed by the successive addition of water, 15% sodium hydroxide, and water. The filtrate was concentrated in vacuo to give 3.8 g of a gum which was purified by chromatography on 300 g of Florisil and eluted with graded mixtures of ether (25–50%)–petroleum ether (bp 30–60°). Following chromatography, there was obtained 2.8 g (57%) of **4b** as an amber glossy solid: NMR δ ($CDCl_3$) 0.85 (t, 3 H, ω-CH_3), 1.05 (s, 3 H, α-CH_3 at C_6), 1.36 (s, 3 H, β-CH_3 at C_6), 1.67 (br s, 3 H, olefinic CH_3), 3.28 (br

d, 1 H, $H_{10\alpha}$), 4.18 (m, 1 H, CHOH), 5.40 (br s, 1 H, vinylic), 6.0, 6.37 (br d, d, 2 H, J = 16 Hz, vinylic side chain), 6.4 (br s, 2 H, aromatic); ir (neat) 3370, 1613, 1572 cm^{-1}. Anal. ($C_{24}H_{34}O_3$) C, H.

On treatment with Ac_2O in pyridine (steam bath, 3 h) **4b** was converted to its diacetate (97% yield): ir (neat) 1770, 1740 cm^{-1}.

Pharmacology. Mice were used in all tests. The MED's for ataxia and increased reactivity to sensory stimuli were determined by administering the drug intravenously (iv) as a solution in 0.06 ml of polyethylene glycol (PEG) 400 per 25-g mouse. Control mice, administered with PEG 400 only, showed a transient mild depression of behavior which disappeared in 2–3 min whereas the drug effects were longer lasting. For oral (po) preparations the drug was given as a suspension in 0.5% methylcellulose. The *mouse fighting test* (foot shock–induced fighting behavior in mice) was carried out for potential tranquilizer activity using a modified Tedeschi procedure described by us earlier.[8] The (±)-*Dopa potentiation test* consisted of determining the potentiation of motor responses to a challenge dose of (±)-Dopa following pretreatment with pargyline and the test compound. The antinociceptive activity was determined using the mouse *hot-plate test*. Both these tests have been described by us previously.[8]

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Cardenolide Analogues. 1. A 17β-Unsaturated Aldehyde

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A 17β-unsaturated aldehyde analogue [3β,14β-dihydroxy-5β-pregn-17β-*trans*-20-en-22-al (**7**)] of the cardenolides was synthesized and studied. In earlier studies by Rappoport, unsaturated aldehydes were found to be highly active electrophiles, more active, for example, than unsaturated nitriles or methyl esters. The synthesis followed in part a scheme previously reported by Thomas for the syntheses of the 17β-unsaturated nitrile **9** and the 17β-unsaturated methyl and ethyl esters **8** and **10**. Both **9** and **8** are more Na^+,K^+-ATPase inhibiting and slightly less inotropic than digitoxigenin (**1b**). However, the unsaturated aldehyde **7** was less Na^+,K^+-ATPase inhibiting (I_{50} = 9.9 ± 0.7 × 10^{-7} M) and less inotropic (100% increase in contractile force at 8.5 ± 1.0 × 10^{-6} M) than **1b** (I_{50} = 4.6 ± 1.6 × 10^{-7} M; 100% increase at 3.0 ± 1.0 × 10^{-7} M).

Structure–activity relationships of the digitalis analogues have recently been reviewed.[1-3] Irrespective of the pharmacological mechanisms operating, several models have been proposed to describe the role of the lactone ring in cardenolide–receptor binding. Two which account quite well for biological data are (1) a Michael attack by Na^+,K^+-ATPase on the unsaturated lactone ring;[4] and (2) ionic binding at the β carbon of the unsaturated lactone system and at the oxygen of the lactone carbonyl.[1] Since the strength of the dipole between these two atoms also determines reactivity in Michael reactions, the two mechanisms are quite similar. That is, sterically unhindered substrates possessing a reactive, polarizable π-cloud system should bind well.

Rappoport and co-workers[5] have recently reported that α,β-unsaturated systems of general structure CH_2=CHX have the following Michael reactivity order in vitro: PhCO > SO_3Ph > CHO > CH_3CO > COOPh > p-CH_3PhSO_2 > $COOCH_3$ > CN > $CONH_2$ > $PO(OEt)_2$ > p-NO_2Ph. For example, in the addition of morpholine to the olefin in absolute methanol at 30°, k_{rel} (X=CHO) = 123, k_{rel} ($COOCH_3$) = 1.0, k_{rel} (CN) = 0.53. Based on the previously discussed models, one might anticipate that cardenolide analogues differing only in the nature of the 17β-unsaturated side chain might approximate the Rappoport order. Thomas has found that the unsaturated nitrile **9** and methyl ester **8** are slightly more Na^+,K^+-ATPase inhibiting than digitoxigenin (**1b**) and slightly less inotropic.[6] We therefore thought it would be interesting to employ the most reactive substrate in the Rappoport series, i.e., the α,β-unsaturated aldehyde. [While the PhCO and SO_3Ph derivatives were more reactive in the Rappoport studies, the bulk of the phenyl group would be expected to negate any activity in cardenolides analogous to **9** and **8**. For example, Thomas has found that the ethyl ester **10** has essentially no Na^+,K^+-ATPase inhibitory nor