cool slowly to room temperature with stirring. After overnight refrigeration, 0.42 g (72%) of a maroon-colored amorphous solid was collected: mp 225–226 °C dec; UV (95% EtOH) λ_{max} 312.5 nm (ϵ 24 950), 504 (5200). The analytical sample was stirred overnight with 1% (v/v) HCl, collected via suction, and washed at the filter with cold portions of deionized, distilled water and methanol. Anal. ($C_{13}H_{16}N_6S_3Cu$) C, H, N.

Biological Testing. A. Liver Slice. The procedure employed in obtaining rat liver slices of appropriate size has been described in some detail in a previous publication.[6] Methods for monitoring the effect of the several chelates on the respiration rate of the liver slices have similarly been discussed.

B. Ascites. The procedure employed in maintaining and harvesting the Ehrlich ascites tumor cells from Swiss white mice has been described in a previous publication.[6] Methods for monitoring the inhibitory effect of the copper chelates on the respiration rates of the ascites cell suspension have also been described earlier.

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Notes

Drugs Derived from Cannabinoids. 7.[1] Tachycardia and Analgesia Structure–Activity Relationships in Δ^9-Tetrahydrocannabinol and Some Synthetic Analogues

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Δ^9-Tetrahydrocannabinol (Δ^9-THC) and eight other synthetic analogues were found to induce a dose-related increase in heart rate in the conscious Wistar rat. In a comparison of tachycardia with analgesic activity (mouse hot-plate and antiwrithing tests) it was found that the water-soluble ester derivatives of **2a**, 1-hydroxy-3-(3-methyl-2-octyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran (DMHP), had the least potency for tachycardia and the greatest potency for analgesia. These findings suggest that these compounds may have promise as therapeutic agents.

Compounds structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC), the major active constituent of marihuana (cannabis sativa), are known to have analgesic properties.[2,3] In man, however, one of the most consistently observed physiological effects of these agents is tachycardia,[4,5] which would tend to reduce their potential usefulness as therapeutic agents. Heretofore, tachycardia activity has been difficult to assess because in the usual laboratory animal preparations cannabinoids induce a decrease in heart rate.[5] Recently, however, we found that in the conscious rat Δ^9-THC and other cannabinoids led to tachycardia similar to that seen in man[6,7] and further that the potency for this effect was generally less in the phenolic ester derivatives of Δ^9-THC than in the parent compound.[7-9]

Many of these compounds had been previously tested for antinociceptive activity in mice after oral administration and a number of them found to be more potent than codeine, propoxyphene (Darvon), and a narcotic analgesic, anileridine;[3a] in addition, several of the more

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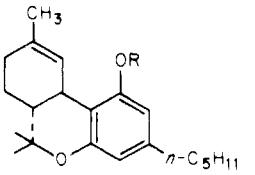
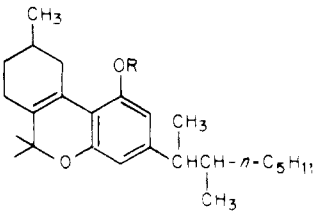
Table I. Tachycardia (Rats) and Analgesic Activity (Mice) of Δ^9-THC and a Water-Soluble Derivative



compd	R	rat, tachycardia, ED_{50}, mg/kg[a] ip[d]	mouse: hot plate, ED_{50}, mg/kg[b] po[d]	mouse: antiwrithing, ED_{50}, mg/kg[c] po[d]
1a (Δ^9-THC)	H	0.062 (0.032-0.120)	>100	43 (31.5-59.6)
1b	-C(=O)$(CH_2)_3$-c-N$(CH_2CH_2)_2$O·HBr	0.092 (0.047-0.172)	168 (72-390)	>40

[a] Δ^9-THC (1a), 12 rats at each dose; compound 1b, five rats at each dose. [b] Ten mice at each dose for each compound. [c] Five mice at each dose for each compound. [d] 95% confidence limits in parentheses.

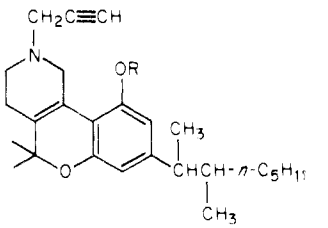
Table II. Tachycardia (Rats) and Analgesic (Mice) Activity of DMHP (2a) and Its Water-Soluble Derivatives 2b and 2c



compd	R	rat, tachycardia, ED_{50}, mg/kg[a] ip[e]	mouse: hot plate, ED_{50}, mg/kg[b] po[e]	mouse: antiwrithing, ED_{50}, mg/kg[c] po[e]
2a (DMHP)[d]	H	0.31 (0.13-0.73)	9.3 (4.3-20.2)	3.0 (0.8-6.6)
2b	-C(=O)$(CH_2)_3$-c-NC_5H_{10}·HCl	1.92 (0.99-3.74)	15.0 (6.6-34.1)	25.9 (18.6-38.9)
2c	-C(=O)$(CH_2)_3$-c-N$(CH_2CH_2)_2$O·HBr	2.13 (0.77-5.86)	0.5 (0.1-1.3)	2.3 (0.9-4.8)

[a] DMHP (2a), six rats at each dose; compounds 2b and 2c, five rats each dose. [b] All compounds ten mice at each dose. [c] All compounds five mice at each dose. [d] The erythro/threo ratio was approximately 66:34 (GLC). [e] 95% confidence limits in parentheses.

Table III. Tachycardia (Rats) and Analgesic (Mice) Activity of Compound 3a and Its Water-Soluble Derivatives 3b-d



compd	R	rat, tachycardia, ED_{50}, mg/kg[a] ip[e]	mouse: hot plate, ED_{50}, mg/kg[b] po[e]	mouse: antiwrithing, ED_{50}, mg/kg[c] po[e]
3a[d]	H	0.061 (0.015-0.26)	7.7 (4.1-12.7)	4.3 (3.2-5.9)
3b	-C(=O)$(CH_2)_3$-c-NC_5H_{10}·HCl	0.11 (0.046-0.24)	5.3 (1.8-15.8)	12.0 (9.3-16.9)
3c	-C(=O)CH$(CH_3)(CH_2)_2$-c-NC_5H_{10}·2HCl	0.40 (0.23-0.72)	4.6 (1.5-14.2)	12.2 (7.7-33.2)
3d	-C(=O)CH$(CH_3)(CH_2)_2$-c-NC_5H_9-2-CH_3·2HCl	0.53 (0.17-1.65)	3.8 (1.6-8.9)	7.3 (2.7-20.3)

[a] All compounds five rats at each dose. [b] All compounds ten mice at each dose. [c] All compounds five mice at each dose. [d] The erythro/threo ratio was approximately 73:27 (GLC). [e] 95% confidence limits in parentheses.

active agents were water-soluble crystalline salts rather than the intractable oils of the parent cannabinoids. It seemed of interest, therefore, to determine tachycardia and compare this undesirable effect with what may prove to be the useful analgesic activity of some of these agents, particularly in those with greater antinociceptive activity.

Experimental Section

Heart rate was measured in conscious male Wistar rats (Charles River Breeding Laboratories, Wilmington, Mass.). Rats were placed on a warming plate inside a plastic housing (Narco Bio-systems) and a piezo electric contact microphone was taped to the tail. Heart rate from the pulse sound was recorded by means of a Model 7P4 tachograph and a Model 7C recorder (Grass Instruments, Quincy, Mass.). Rectal temperature was measured and kept below 39 °C (for further details of heart rate measurement, see ref 7). Each agent was injected intraperitoneally (ip) in a small volume (≤0.1 mL) of either absolute ethanol or distilled water, depending on its solubility characteristics. Analgesic activity was determined in mice by hot-plate and antiwrithing tests as previously reported.[2,3] The dose required for half-maximal effect (ED_{50}) and its confidence limits for tachycardia and antinociceptive activity for each compound was determined by a computerized modification of the method of Litchfield and Wilcoxon; this method enables one to test the slopes of dose-effect curves for parallelism which was done in the case of those for tachycardia.[10]

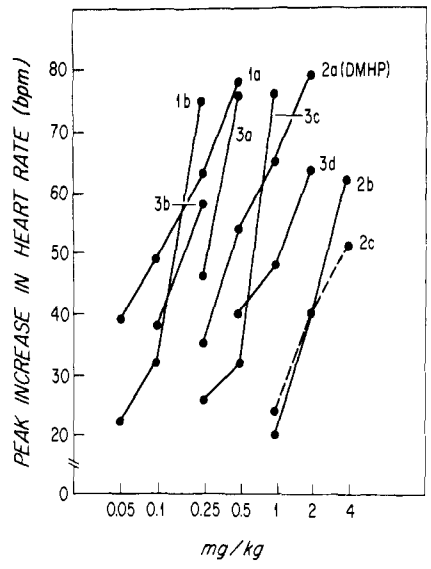


Figure 1. Dose effects for Δ^9-THC and eight other synthetic analogues. Maximum increases in heart rate for each compound are plotted against logarithm of dose.

Results and Discussion

Dose–effect curves for heart rate response in the conscious rat after Δ^9-THC and eight other cannabinoid compounds are presented in Figure 1; there were no statistically significant differences among these slopes.[10] The reference compound, Δ^9-THC, had high potency for tachycardia and low potency for analgesia while its water-soluble 4-morpholinobutyryl ester, **1a**, had similar properties (Table I). The compound 1-hydroxy-3-(3-methyl-2-octyl)-6,6,9-trimethyl-7,8,9,10-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran (DMHP, **2a**), one of the few synthetic derivatives of Δ^9-THC that have been given to human subjects,[11] gave a striking increase in analgesic potency and a moderate decline in potency for tachycardia (Table II) compared to Δ^9-THC. In the rodent,[7] as in man,[11] DMHP induces a more prolonged tachycardia than Δ^9-THC. The water-soluble 4-piperidinobutyryl ester of DMHP (**2b**) had less tachycardia potency but also less analgesic potency (Table II). The 4-morpholinobutyryl ester of DMHP (**2c**), however, had the least potency for tachycardia and highest potency for analgesia of any of the compounds tested (Table II).

In another series of nitrogen analogues of DMHP (**3a–d**) an attempt was made to increase analgesia by incorporating the arylpiperidino structure common to narcotic agents. Although **3a** was an active analgesic, its potency for tachycardia was as great as that for Δ^9-THC (Table III). However, **3b**, a water-soluble ester of **3a**, gave somewhat less tachycardia and retained a fair degree of analgesic potency (Table III). Further modification of the ester carbon chain, as in **3c** and **3d** (Table III), gave progressively less potency for tachycardia and greater potency for analgesia.

Thus, in comparison to Δ^9-THC, the most prominent increases in analgesia and decreases in tachycardia potency were found in the synthetic analogues of DMHP and its water-soluble esters (Table II). Replacing the alicyclic ring of DMHP with the nitrogen-containing tetrahydropyridine ring (e.g., **3a**), brought no great change in analgesia and an increase in tachycardia. The water-soluble esters of both base compounds, however, tended to have greater potency for analgesia with less potency for tachycardia, suggesting that these compounds may have promise as therapeutic agents.

From this study it appears that the esterification of the free phenol in cannabinoids leads to a decrease in tachycardia and this effect may be related to the ease of hydrolysis of these esters, since the least potency for tachycardia was found in the more hindered esters (compare **3d** with **3a**) which hydrolyze less readily to form the parent compound.[9]

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