# Synthesis and Pharmacological Activity of a Phosphate Ester of $\Delta^8$-Tetrahydrocannabinol

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A water-soluble phosphate ester of $\Delta^8$-tetrahydrocannabinol ($\Delta^8$-THC) was synthesized and its pharmacological activities were examined. The cataleptogenic and thiopental sleep-potentiating effects of $\Delta^8$-THC phosphate in the mouse were approximately 10 and 7% of those of $\Delta^8$-THC, respectively. However, this phosphate showed almost the same potency and a longer duration of hypothermic effect, as compared with $\Delta^8$-THC in the mouse. The acute toxicity of this phosphate was far lower than that of $\Delta^8$-THC. $\Delta^8$-THC phosphate was difficultly hydrolyzed by alkaline phosphatase or mouse liver homogenate in vitro. The mode of action of the phosphate derivative is discussed in connection with this enzymatically difficult hydrolysis.

It is generally accepted that the phenolic group of morphine is essential for the production of analgesia. Modifications of this group have invariably resulted in a reduction or complete loss of activity. Recently, however, we have demonstrated that the intracerebral injection of a phosphate ester of morphine induced potent analgesia in mice, similar to the parent drug.[1] This phosphate also significantly inhibited nicotine-induced contraction of guinea pig ileum without a latent period.[2] These findings suggest that the introduction of a phosphate residue into a pharmacologically essential site of a drug does not necessarily interfere with its normal properties. In the case of morphine, the pharmacological effect of the ester was comparable with that of the parent drug.

Application of the above view to $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC), a hallucinative constituent of marihuana, merits consideration. One can expect that this highly lipophilic drug would be modified to a water-soluble, but still pharmacologically active compound. One such derivative, a morpholinobutyrate hydrochloride of $\Delta^9$-THC equivalent in potency to the parent compound, has been synthesized by Zitko et al.[3] However, this ester was easily hydrolyzed and liberated the active moiety in vivo.

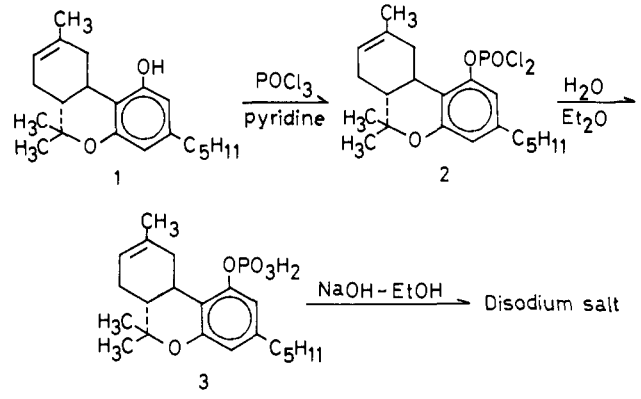
In the present study, we examined the above findings to another drug, $\Delta^8$-THC, chemically more stable than and almost equipotent to $\Delta^9$-THC,[4,5] and utilized $\Delta^8$-THC phosphate as a water-soluble derivative. This communication describes the synthesis of $\Delta^8$-THC phosphate as well as its pharmacological activities including acute toxicity. The enzymatic hydrolysis of this phosphate ester has also been studied in vitro to gain some insight into its mode of action.

## Results

**Synthesis.** The orthophosphate ester of $\Delta^8$-THC was synthesized as outlined in Scheme I. $\Delta^8$-THC (1) was treated with phosphoryl chloride in dry pyridine at 4 °C and the resulting *O*-dichlorophosphoryl-$\Delta^8$-tetrahydrocannabinol (**2**) was purified by high vacuum distillation. This oily product was dissolved in ether and converted to $\Delta^8$-THC phosphate **3** by mild hydrolysis with water. This phosphate ester still possessed high lipid solubility and was obtained from the ether layer. **3** was then transformed to the water-soluble salt **4** by treatment with alcoholic sodium hydroxide. NMR spectra of **2** and **3** indicated that no isomerization of the double bond occurred. However, when $\Delta^9$-THC was used initially, the same procedure afforded a mixture of $\Delta^8$- and $\Delta^9$-THC phosphates.

**Pharmacological Results.** The acute toxicity of $\Delta^8$-THC phosphate was significantly lower than that to $\Delta^8$-THC; the phosphate did not cause death up to a dose of 160 mg/kg iv in the mouse, whereas the $LD_{50}$ of $\Delta^8$-THC was estimated to be 27.5 (23.1–32.7) mg/kg iv.[6]

**Scheme I.** Synthetic Route to $\Delta^8$-THC Phosphate




The peak cataleptogenic effect of $\Delta^8$-THC phosphate was attained 60 min after injection, while that of $\Delta^8$-THC at about 30 min after. At these peak times, the $ED_{50}$ of $\Delta^8$-THC [3.3 (1.9–5.6) mg/kg] was approximately one-tenth of that of $\Delta^8$-THC phosphate [37.0 (21.8–62.9) mg/kg].[6]

$\Delta^8$-THC phosphate was less potent and had a more delayed onset of effect than $\Delta^8$-THC in potentiating thiopental-induced sleep. The effect of $\Delta^8$-THC phosphate (in saline) was maximal at 60 min after injection, while that of $\Delta^8$-THC (in 1% Tween 80) was highest at 30 min. At these peak times, the $ED_{50}$ of $\Delta^8$-THC [1.2 (0.7–2.2) mg/kg] was $^1/_{15}$th of that of $\Delta^8$-THC phosphate [17.5 (9.5–32.3) mg/kg].[6] The mean sleeping time of controls pretreated with saline was 7.9 ± 0.6 min and that of controls given 1% Tween 80 solution 6.9 ± 0.6 min.[7]

Contrary to above reduced activities, the hypothermic effect of $\Delta^8$-THC phosphate was almost equipotent to that of $\Delta^8$-THC in mice at a dose of 20 mg/kg, as shown in Figure 1, although the former was a little less potent at 10 mg/kg. It should also be noted that the effect of the phosphate was delayed slightly in onset and, yet, longer in duration than that of the parent compound. The decline in rectal temperature (mean ± SE of eight mice) was respectively 2.4 ± 0.5 and 5.0 ± 0.3 °C for 10 and 20 mg/kg 2 h after iv injection of $\Delta^8$-THC phosphate and 3.8 ± 0.3 and 4.8 ± 0.3 °C at the same doses 1 h after iv injection of $\Delta^8$-THC.

**Enzymatic Hydrolysis of $\Delta^8$-THC Phosphate.** $\Delta^8$-THC phosphate could be hydrolyzed enzymatically, but the affinity to the enzyme did not appear to be high (see Table I). $\Delta^8$-THC (12%) was liberated from the phosphate by hydrolysis with alkaline phosphatase at the optimum pH of 9.6 for 60 min. As summarized in Table I, when incubated with the mouse liver homogenate for 60 min, 24.5 and 29.2% of the phosphate was hydrolyzed at pH 9.6 and 5.0, respectively. Contrary to these results, *p*-nitrophenyl phosphate was hydrolyzed almost com-

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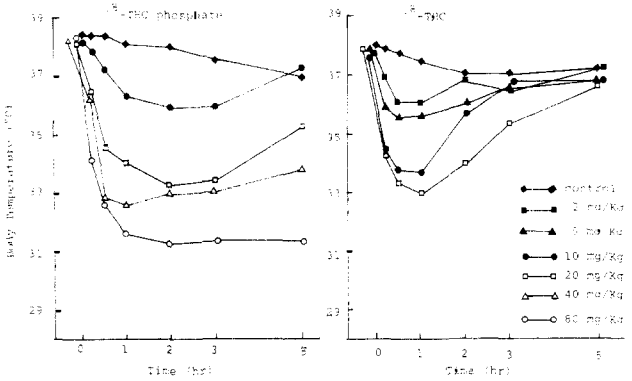

**Figure 1.** Time course of changes in body temperature produced by $\Delta^8$-THC phosphate and $\Delta^8$-THC in mice.

Table I. Percent Hydrolysis of $\Delta^8$-THC Phosphate and *p*-Nitrophenyl Phosphate by Mouse Liver Homogenate

| incubation pH | substrate | % hydrolysis[a] 15 min | 30 min | 60 min[b] |
|---|---|---|---|---|
| 9.6 | $\Delta^8$-THC phosphate | 7.8 | 13.9 | 24.5 |
| | *p*-nitrophenyl phosphate | 21.5 | 36.4 | 73.6 |
| 5.0 | $\Delta^8$-THC phosphate | 15.3 | 20.7 | 29.2 |
| | *p*-nitrophenyl phosphate | 88.9 | 96.7 | 97.7 |

[a] The values are expressed as the mean of duplicate determinations. [b] Incubation time.

pletely by the liver homogenate in the acidic medium (pH 5.0) after 30–60 min of incubation. Hydrolysis of this phosphate proceeded somewhat more slowly in the alkaline medium (pH 9.6).

## Discussion

$\Delta^8$-THC phosphate was synthesized in the hope of obtaining a water-soluble derivative maintaining the same pharmacological activity as $\Delta^8$-THC. However, the phosphate exhibited only about 10 and 7% of the activity of the parent compound for cataleptogenic effect and thiopental-sleep potentiation, respectively. Furthermore, the effect of $\Delta^8$-THC phosphate attained maximum levels later than those of $\Delta^8$-THC. These results could be readily explained if one assumed either that the phosphate itself was pharmacologically inactive, but the parent compound was liberated slowly and transformed into its psychoactive hydroxylated metabolites in vivo, or that the phosphate itself possessed lower, but distinct activity. The very slow in vitro hydrolysis and diminished acute toxicity of this phosphate support both of these views. On the other hand, the hypothermic effect of $\Delta^8$-THC phosphate in mice was almost equipotent to and longer in duration than that of $\Delta^8$-THC at a dose of 20 mg/kg. This potency of the phosphate appears to be too high to attribute exclusively to the action of $\Delta^8$-THC being slowly liberated from the phosphate. Therefore, it seems more plausible to assume that the phosphate itself possesses a considerably potent hypothermic effect. These findings are particularly interesting because they indicate the possibility that a phosphate residue introduced into a pharmacologically essential site of a drug molecule does not always interfere with the action of the drug, even if the phosphate residue is difficult to remove in vivo. Additionally, the present results also suggest that the introduction of a phosphate group may be a useful way to modify drugs which otherwise exert some undesirable side effects.

## Experimental Section

Microanalyses were performed on a Yanagimoto MP-2 CHN corder and were within 0.4% of theoretical values. IR and NMR spectra were respectively recorded on a Nihonbunko DS-701G spectrophotometer and a JEOL PS-100 spectrometer using $Me_4Si$ as the internal standard. Mass spectra were recorded on a JEOL-01SG spectrometer.

**$\Delta^8$-THC (1).** 1 was synthesized from $\Delta^9$-THC, isolated from *Cannabis sativa*, by isomerization according to the method of Gaoni and Mechoulam.[5]

***O*-Dichlorophosphoryl-$\Delta^8$-tetrahydrocannabinol (2).** To 552 mg of 1 dissolved in 1.5 mL of dry pyridine and cooled in ice, 450 mg of phosphoryl chloride was added. After allowing to stand overnight at 4 °C, the reaction mixture was concentrated under reduced pressure and extracted twice with 3-mL portions of dry benzene. The solvent was evaporated off and the remaining residue was distilled at 160–170 °C (bath temperature) (0.01 mmHg). The colorless distillate was redistilled similarly to yield 486 mg of *O*-dichlorophosphoryl-$\Delta^8$-tetrahydrocannabinol (2): mass spectrum *m/e* 430 ($M^+$); NMR ($CDCl_3$) $\delta$ 6.72 (d, $J$ = 2 Hz, ArH, 1), 6.60 (d, $J$ = 2 Hz, ArH, 1), 5.45 (br m, $C_8$-H, 1), 2.52 (t, $ArCH_2$, 2), 1.72 (s, $C_9$-$CH_3$, 3), 1.41 (s, *gem*-$CH_3$, 3), 1.10 (s, *gem*-$CH_3$, 3), 0.88 (t, $\omega$-$CH_3$, 3).

**$\Delta^8$-THC Phosphate (3).** To a solution of 2 in 50 mL of $Et_2O$, 30 mL of distilled water was added and the mixture was stirred for 15 h at 4 °C. The $Et_2O$ layer was washed with distilled water, dried, and evaporated to yield 398 mg of $\Delta^8$-THC phosphate (3): mass spectrum *m/e* 394 ($M^+$); NMR ($CDCl_3$) $\delta$ 10.72 (br s, $PO_3H_2$, 2), 6.67 (d, $J$ = 2 Hz, ArH, 1), 6.54 (d, $J$ = 2 Hz, ArH, 1), 5.40 (br m, $C_8$-H, 1), 2.50 (t, $ArCH_2$, 2), 1.67 (s, $C_9$-$CH_3$, 3), 1.36 (s, *gem*-$CH_3$, 3), 1.06 (s, *gem*-$CH_3$, 3), 0.86 (t, $\omega$-$CH_3$, 3).

**Disodium $\Delta^8$-THC Phosphate (4).** 3 was treated with 2 equiv mol of alcoholic sodium hydroxide and 348 mg of the disodium salt was precipitated as a hygroscopic powder by adding dry $Et_2O$ to the resulting solution. Twenty milligrams of this powder could easily be dissolved in 1 mL of 0.9% NaCl solution: UV spectrum (95% EtOH) $\lambda_{max}$ 275 nm (log $\epsilon$ 3.18), 283 (3.21); IR spectrum (KBr) 1235 (POC), 1080 $cm^{-1}$ (P=O). Anal. ($C_{21}H_{29}O_5PNa \cdot 2.5H_2O$) C, H, P.

**Pharmacological Experiments.** Male mice (ddN strain), 20–25 g, were used. Drugs were injected iv through the tail vein (0.1 mL/10 g of body weight), as a suspension in 1% Tween 80 ($\Delta^8$-THC) or as a solution in saline (disodium $\Delta^8$-THC phosphate and sodium thiopental) to eliminate the factor of penetration from the site of administration to the circulation. For each dose of the drug, eight animals were used. Controls were given the vehicle only.

$LD_{50}$ and its 95% confidence limits were calculated by the method of Litchfield and Wilcoxon.[8] Mortality was determined 24 h after the injection. Catalepsy was assessed by a modification of the simple bar test used by Simon et al.[9] at 30 and 60 min after iv injection of $\Delta^8$-THC and $\Delta^8$-THC phosphate. The bar test was carried out by placing the front paws of the mouse on a horizontal metal bar (0.5 cm in diameter), 6 cm in height, and forcing the mouse to stand on its hind legs. If the mouse maintained this abnormal position for more than 30 s, catalepsy was regarded to be positive. The effect of the phosphate was compared with that of the parent compound at the respective time of peak effect.

The effect of $\Delta^8$-THC and $\Delta^8$-THC phosphate on sleep induced by iv injection of 80 mg/kg of thiopental was examined at 30 and 60 min after pretreatment with the test drugs. Loss of the righting reflex was used as an index of sleep. If the sleeping time of the treated animals was prolonged more than twice the respective control values, potentiation was regarded to be positive. Controls were pretreated with saline (vehicle for $\Delta^8$-THC phosphate) or a 1% Tween 80 solution (vehicle for $\Delta^8$-THC). The rectal temperature was measured using a thermistor thermometer (Nihon Kohden). Room temperature was maintained at 23 ± 1 °C. The time course of body temperature was plotted up to 5 h after treatment.

**Enzymatic Hydrolysis of $\Delta^8$-THC Phosphate.** 4 (1.5 $\mu$mol) was incubated with 1.5 units of alkaline phosphatase (Sigma Chemical Co.) in 0.1 M glycine buffer (pH 9.6) or with liver homogenate of mice (equivalent to 0.1 g of liver) in the same buffer or 0.1 M acetate buffer (pH 5.0) at 37 °C for 1 h. The reaction was terminated by addition of 1.0 mL of 2% phosphotungstic acid in 0.1 N HCl. The liberated $\Delta^8$-THC was extracted three times with 5-mL portions of *n*-hexane, concentrated, and determined by a Yanagimoto Model G-8 gas chromatograph equipped with

a hydrogen flame ionization detector and a 1.5 m × 3 mm i.d. glass column containing 1% XE-60 on 60–80 mesh Chromosorb W at 210 °C.

*p*-Nitrophenyl phosphate (Sigma Chemical Co.), used as the reference compound for enzymatic hydrolysis, was incubated by the same procedure as $\Delta^8$-THC phosphate. The liberated *p*-nitrophenol was assayed as follows. The incubation mixture was centrifuged for 10 min at 2000 rpm after the addition of 5 mL of water. To 2.0 mL of the supernatant, 1 mL of 2 N KOH was added and the final volume was adjusted to 10 mL with water. *p*-Nitrophenol in this solution was then determined spectrophotometrically at 400 nm.

## References and Notes


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(6) The values in parentheses show 95% confidence limits.
(7) The values show mean ± SE (min) of sleeping time in eight mice.
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# 2-Methoxyphenylethanolamines, Potential β-Adrenergic Blocking Agents


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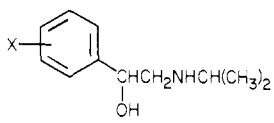
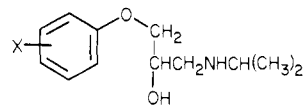
*Received February 10, 1978*


The effect of the introduction of a 2-methoxy substituent on the β-adrenergic antagonistic properties of a series of 3- and 4-substituted phenylethanolamines (**1**) was studied. Both the series of bromo- and methyl-substituted compounds behaved similarly, indicating that electronic forces are not significant in determining β-adrenergic antagonist activity. When compared with the corresponding phenylethanolamines without a 2-methoxy substituent, the 2-methoxy-4-substituted derivatives (**3a** and **3d**) had enhanced potency and selectivity but the 2,3- (**3b** and **3e**) and the 2,5-disubstitution patterns (**3c** and **3f**) showed a loss of activity. The inconsistent changes in activity prevented any firm conclusions being made about the effect of the ether oxygen and the β-adrenoceptor antagonistic activity of phenoxypropanolamines.


Two distinct chemical classes may exert action as antagonists of β-adrenoceptors. The 1-phenylethanolamines 1 resemble closely the structure of the natural biogenic catecholamines,[1] while the 1-alkylamino-3-aryloxy-2-propanols **2** (aryloxypropanolamines) contain an oxy-




**1**




**2**

**a**, X = 2-OMe
**b**, X = 4-Br
**c**, X = 3-Br
**d**, X = 5-Br
**e**, X = 4-$CH_3$
**f**, X = 3-$CH_3$
**g**, X = 5-$CH_3$

methylene group inserted between the aryl ring and the ethanolamine side chain.[2]

The similarity of β-adrenoceptor antagonistic action may be due to the ability of the phenoxypropanolamines to assume a conformation about the oxymethylene bonds (**2**) so that when the benzene rings of models of phenethanolamines 1 and phenoxypropanolamines **2** are aligned the ethanolamine side chains may assume conformations which are superimposable.[3]

Recently an alternative bicyclic rigid conformation involving intramolecular hydrogen bonding between the protonated nitrogen substituent and the ether and alcoholic oxygens has been proposed to account for the similar activity of the two classes.[4]

Although either suggestion may help to account for the similarity of action of the two classes of β-adrenergic blocking agents, they do not explain the general observation that the phenoxypropanolamines are often more potent and selective for the $\beta_1$-adrenoceptors than the phenylethanolamines. We considered that the phenoxypropanolamines had the possibility of additional bonding between the extra ether oxygen and the β-adrenoceptor site, and this may in some way contribute to the extra activity and selectivity. To test this we have prepared for comparison of their β-adrenergic blocking properties a series of phenylethanolamines with and without a 2-methoxy group.

These 2-methoxyphenylethanolamines have the possibility of extra interaction with the β-adrenoceptor site and have potential as potent and $\beta_1$-selective blocking agents, as they appear to be able to adopt a conformation where all the essential features of the phenoxypropanolamines are available for interaction with the receptor sites.

In the present article are reported the synthesis and results of preliminary pharmacological studies of a series of 2-methoxyphenylethanolamine derivatives.

**Chemistry.** Both the 2-methoxy-5-substituted and the 2-methoxy-4-substituted phenethylamines **3** were prepared from the appropriate acetophenones **4** which were obtained by Fries rearrangement of the acetates[5-7] (see Scheme I).

The acetophenones were converted into the epoxides **5** by bromination of the side chain, reduction of the ketone, and reaction of the bromohydrin with base.

The 2-methoxy-3-substituted phenethylamines were prepared from the corresponding aldehydes **6** as our Fries rearrangement of *o*-bromo acetate gave rise to multiple products.[8] Our o-hydroxyaldehydes were prepared conveniently but in low yields by the Duff reaction.[9]

The appropriate 2-methoxy-3-substituted benzaldehydes (**5**, Z = Br, $CH_3$) were obtained by methylation of the

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