Structure–Activity Studies on Tetrahydro- and Hexahydrocannabinol Derivatives

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Abstract □ A series of tetrahydro- and hexahydrocannabinol derivatives was prepared in which the substituents at position 9 were varied. These compounds were evaluated in mice for their effects on locomotor activity, body temperature, muscle tone, and analgesia. Depression of body temperature and locomotor function was demonstrated by several compounds, but all were devoid of any significant analgesic activity.

Keyphrases □ Tetrahydrocannabinol derivatives, various—synthesized, pharmacological activity evaluated in mice □ Hexahydrocannabinol derivatives, various—synthesized, pharmacological activity evaluated in mice □ Structure–activity relationships—various tetrahydrocannabinol and hexahydrocannabinol derivatives, pharmacological activity evaluated in mice

Numerous synthetic tetrahydrocannabinol derivatives have been prepared and tested since the accidental discovery that synthetic $\Delta^{6a,10a}$-tetrahydrocannabinol caused ataxia in dogs and corneal areflexia in rabbits (1, 2). Reviews of those studies have been published (3, 4).

The purpose of this study was to examine the structure–activity relationships in some synthetic tetrahydro- and hexahydrocannabinol derivatives with variations in ring C, especially at position 9. A modified primary mouse screening test, developed in this laboratory, was used for the bioassay.

RESULTS AND DISCUSSION

Chemistry—The key intermediate in the synthesis of most of the compounds was the ketone, 9-nor-9-oxohexahydrocannabinol (I, Scheme I). It was prepared either as a racemic mixture according to the procedure of Fahrenholtz *et al.* (5) or as an optically active compound with the natural configuration according to a slightly improved procedure (6). (−)-$\Delta^{9,11}$-Tetrahydrocannabinol acetate[1] was treated with osmium tetroxide and sodium periodate in tetrahydrofuran–water to give the ketone after saponification of the acetate in an overall yield of 75%.

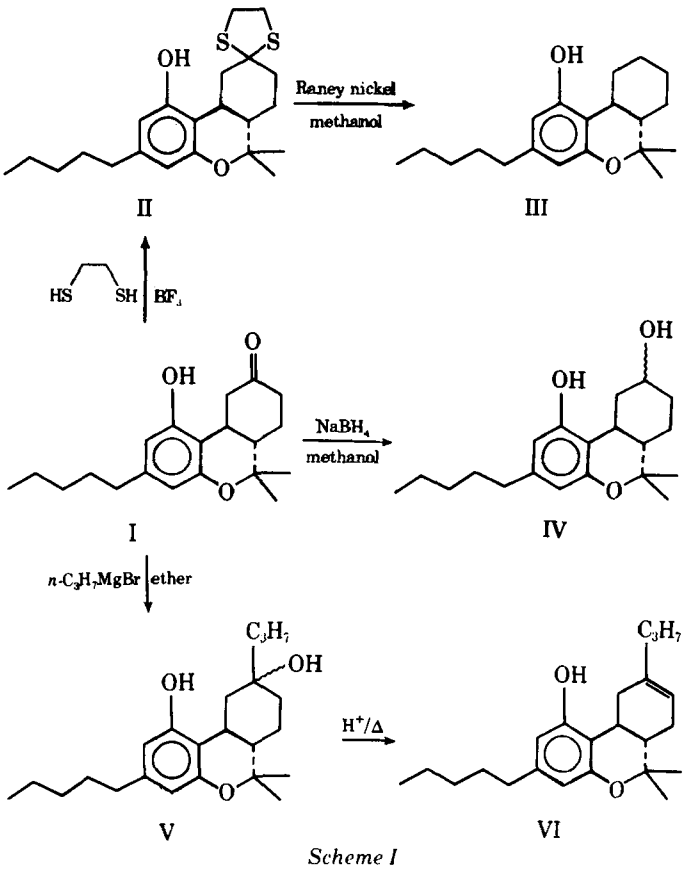
Compound I was converted to the dithioketal (II) by treatment with ethanedithiol–boron trifluoride etherate (7), and it gave 9-norhexahydrocannabinol (III) after reduction with Raney nickel in refluxing methanol (8). The optically active I afforded an approximate 1:1 mixture of the two isomeric 9-nor-9-hydroxyhexahydrocannabinols (IV) in almost quantitative yield by reduction with sodium borohydride in methanol (6).

Grignard reaction with the I acetate and propylmagnesium bromide gave, after treatment with acid, *dl*-Δ^{8}-9-nor-9-*n*-propyltetrahydrocannabinol (VI).

Hydrogenation of (−)-Δ^{9}-tetrahydrocannabinol[1] (VII, Scheme II) with palladium-on-charcoal (10%) yielded an approximately 1:1 mixture of the two isomeric (−)-hexahydrocannabinols (VIII) (9).

Reaction of (−)-$\Delta^{9,11}$-tetrahydrocannabinol acetate (IX, Scheme III) with borohydride–tetrahydrofuran–diglyme and subsequent oxidation of the resulting organoborane with trimethylamine *N*-oxide dihydrate (10) gave, after saponification of the acetate (X), an approximately 1:1 mixture of the two isomeric (−)-11-hydroxyhexahydrocannabinols (XI).

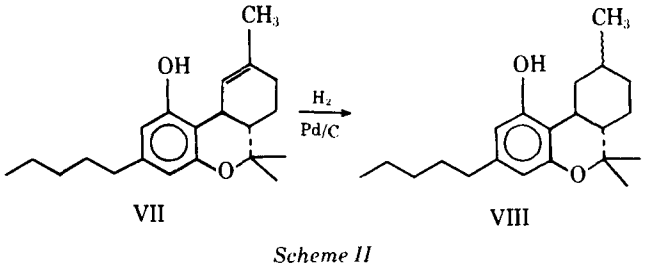
Pharmacology—A primary mouse screening test was applied to bioassay Δ^{9}-tetrahydrocannabinol and its analogs. A battery of 11 relatively objective subtests was administered successively to each mouse in a systematic order. The assessment began by observing the undisturbed behavior and the eyes of the animal to detect any gross abnormality. Then the mouse was put in an open-field box (11) to record its locomotor activity. Postural arrest was noted when the mouse stopped abruptly and remained still for more than 10 sec.



Scheme I

Touch response was evaluated by watching the "popcorn" reaction of the animal when its thorax was touched with the tip of a long forceps. A sharp sound was emitted by means of a metal clicker to score the startle response. To test for the biting response, the animal was held at the nape and a small wooden dowel was brought near its mouth (12). Body temperature was registered by inserting a rectal probe connected to a telethermometer.

Muscle tone was assayed by allowing the animal to suspend from a tiny bar fixed to a 0.5-kg push–pull strain gauge and slowly pulling the mouse down by its tail. The amount of force required to compel the subject to release its grip was read from the gauge. Neuromuscular coordination



Scheme II

[1] Supplied by the Center for Studies of Narcotics and Drug Abuse, Alcohol, Drug Abuse, and Mental Health Adminstration.

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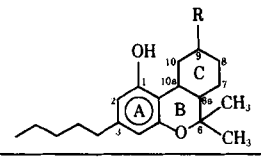
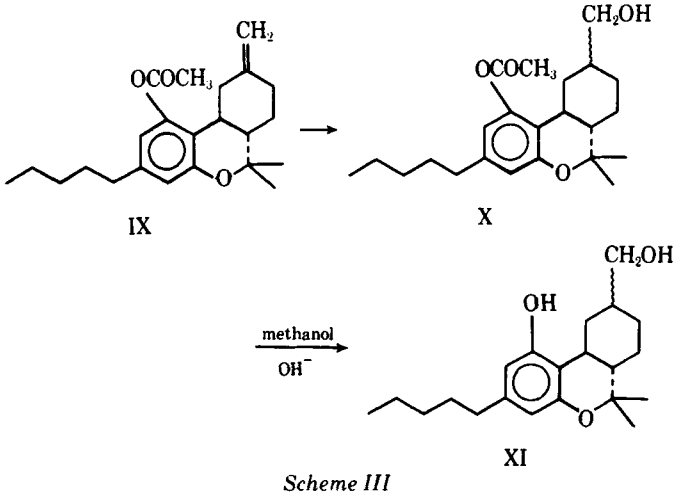


Table I—Structure–Activity Relationships of Δ^9-Tetrahydrocannabinol (VII) and Its Synthetic Analogs in Primary Mouse Screen

Compound	R	Δ	ED_{50}, μm/kg Locomotor Activity	Postural Arrest	Body Temperature	Pain Sensitivity
VII	CH_3	9 (1)	0.8	1.3	1.7	3.2
III	H	—	0.4	1.5	0.9	—
VI	$CH_2CH_2CH_3$	8 (6)	1.6	1.5	17.6	—
VIII	CH_3	—	27.1	21.0	12.7	—
IV	OH	—	0.4	1.1	0.7	—
XI	CH_2OH	—	0.8	1.0	2.0	—



Scheme III

was measured by a rotarod (13). In the analgesic test, the animal was placed on a hot plate (14) regulated at 55 ± 0.5°. The interval of time during which the animal remained on the plate without showing any obvious reaction to heat was recorded.

Swiss–Webster male mice, 18–22 g, were used. Baseline scores for several subtests, *i.e.*, locomotor activity, postural arrest, touch response, biting response, and neuromuscular coordination, were obtained as a basis of comparison between predrug and postdrug responses. Initially, a series of dose range studies was conducted using a $\frac{1}{2}$ $\log_{10}$ interval scale. After a minimum effective dose range was established and a probable slope was estimated for each compound, dose–response studies were performed. The compounds were dissolved in polyethylene glycol 400 and administered at the rate of 2.4 ml/kg ip in a manner similar to the procedure reported by Uliss *et al.* (15).

Three appropriate doses of each compound were selected, and 10 animals were tested at each dose level. Each time a compound was evaluated, a control group of 10 mice injected with polyethylene glycol 400 also was observed. The bioassay was conducted 30 min after treatment.

The predrug baseline scores and the data obtained by assessing the physiological and behavioral states of the control subjects were analyzed to establish a control or criterion value for each subtest. The subtest score of each treated animal was considered to show a decrease, no change, or an increase in response relative to the control value.

The data showed that all compounds decreased locomotor activity and body temperature and increased postural arrest in a substantial number of animals. Although most compounds had no analgesic effects, Δ^9-tetrahydrocannabinol reduced pain sensitivity in some subjects. On other physiological states and behavioral responses, the compounds produced no appreciable effects. The percentages of mice showing a decrease in locomotor activity were computed at each dose level and plotted against dose (in micromoles per kilogram) on logarithmic probability paper. Similarly, the percentages of subjects manifesting a decrease in body temperature and the percentages revealing an increase in postural arrest were plotted against dose.

The dose–response curves indicated that the effects were dose related. From the curves, the median effective doses (ED_{50}'s) were calculated according to the method of Litchfield and Wilcoxon (16). The ED_{50} is the estimated dose at which 50% of the animals are expected to show a decrease (or an increase) in response to a given subtest. The ED_{50} values are listed in Table I, depicting the structure–activity relationships of the most active compounds.

EXPERIMENTAL

Boiling points are uncorrected. Melting points[2], determined in open glass capillaries, are uncorrected. Analytical results for the elements were within ±0.4% of the theoretical values.

***dl*-9-Norhexahydrocannabinol (III)**—Ethanedithiol (3 ml), I (4 g), and 3 ml of boron trifluoride etherate were mixed and stirred until they were homogeneous. After standing for an additional 10 min, this solution was chromatographed on silica gel with methylene chloride–ether. The methylene chloride–2% ether and methylene chloride–5% ether fractions were evaporated to give 4.7 g (95%) of the dithioketal as a colorless oil, which soon started to crystallize. Recrystallization from pentane gave colorless crystals, mp 84–87°.

These crystals were dissolved in 1100 ml of methanol and refluxed for 30 hr with 70 g of Raney nickel. The solution was then filtered over diatomaceous earth[3], and the solvent was evaporated to give 3.4 g (94%) of a yellow oil. Chromatography on silica gel with benzene and subsequent distillation at reduced pressure yielded 2.3 g of a slightly yellow oil, bp 160–180°/0.25 mm.

Anal.—Calc. for $C_{20}H_{30}O_2$: C, 79.42; H, 10.00. Found: C, 79.53; H, 10.32.

***dl*-Δ^8-9-*n*-Propyltetrahydrocannabinol (VI)**—Compound I acetate (800 mg, 2.25 mmoles) was treated with propylmagnesium bromide according to the standard Grignard reaction procedure to afford 600 mg of the mixture of carbinols (V) as a semisolid oil (74%). This crude product was treated with *p*-toluenesulfonic acid in refluxing benzene and chromatographed on silica gel with benzene to give 430 mg (55% overall yield) of VI as a nearly colorless oil.

Anal.—Calc. for $C_{23}H_{34}O_2$: C, 80.65; H, 10.01. Found: C, 80.51; H, 9.75.

11-Hydroxyhexahydrocannabinol (XI)—(−)-$\Delta^{9,11}$-Tetrahydrocannabinol acetate (456 mg, 1 mmole), prepared from (−)-$\Delta^{9,11}$-tetrahydrocannabinol by reaction with acetic anhydride–pyridine, was dissolved in 4 ml of dry diglyme and hydroborated with 0.44 ml of 1 *N* borohydride–tetrahydrofuran solution in tetrahydrofuran at 0° for 1 hr. Then the reaction was refluxed for 2 hr, and trimethylamine *N*-oxide dihydrate was added. The reaction mixture was gently refluxed with stirring for 2 hr. Then the contents of the reaction flask were transferred to a separator, diluted with methylene chloride, and washed once with 10% HCl and three times with saturated sodium chloride solution.

The organic layer was dried (magnesium sulfate) and evaporated to afford 400 mg of a brownish, viscous oil. Saponification of the acetate with methanolic 2% KOH at room temperature overnight and repeated chromatography of the product on silica gel with petroleum ether–40% ether gave 80 mg (25% overall yield) of an approximately 1:1 mixture of the two isomeric carbinols as a yellow oil.

Anal.—Calc. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.76; H, 9.53.

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Synthesis of 4-Substituted Aminobenzoate Quaternary Salts as Potent Antispasmodic Agents

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Received May 30, 1978, from the *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria, Egypt.* Accepted for publication August 24, 1978.

Abstract □ *N*-(Diethylaminoethyl)-4-substituted aminobenzoate quaternary salts, *N*-(diethylaminoethyl)-4-substituted aminobenzamide quaternary salts, 4-substituted acylaminobenzamide quaternary salts, and 4-substituted acylaminosalicylamide derivatives were prepared and tested for antispasmodic activity. Preliminary pharmacological tests on isolated guinea pig ileum revealed that the new compounds possess nonspecific inhibitory action on smooth muscles.

Keyphrases □ Aminobenzoic acid derivatives—quaternary salts synthesized, antispasmodic activity evaluated □ Quaternary salts—of aminobenzoic acid derivatives synthesized, antispasmodic activity evaluated □ Antispasmodic activity—quaternary salts of various aminobenzoic acid derivatives evaluated □ Structure–activity relationships—quaternary salts of aminobenzoic acid derivatives evaluated for antispasmodic activity

Studies based on the empirical structural scission of the atropine molecule indicated that it did not have a highly specific action. The tropine moiety is a complex amino alcohol that can be simplified but still retain the antispasmodic activity.

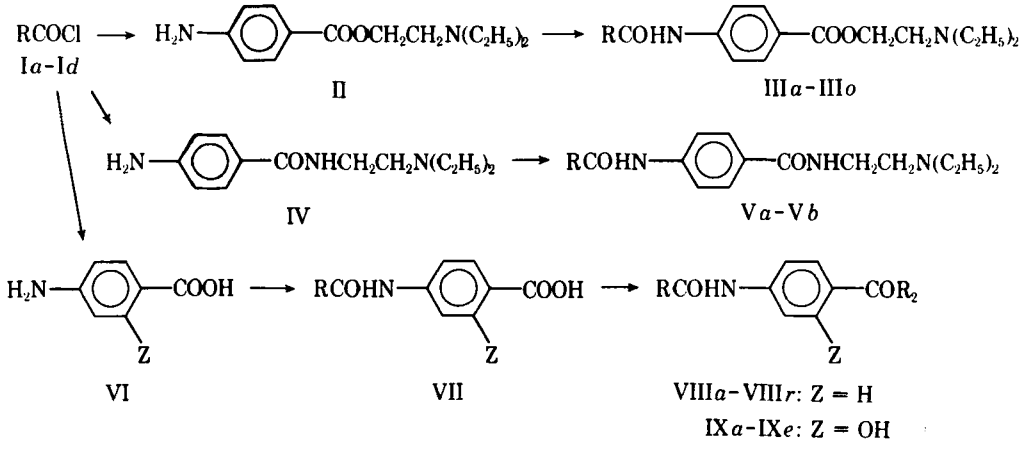
Many parasympatholytic drugs possess potent antispasmodic activity and show some structural resemblance to acetylcholine; at least a tertiary nitrogen atom is needed for activity. Moreover, quaternization of some antispasmodics resulted in significant variations in activity, including enhancement, duration, and toxicity.

The discovery of smooth muscle relaxant properties among many *N*-substituted 4-aminobenzoate esters and amides (1, 2) inspired the preparation of some analogs derived from 4-aminobenzoic and 4-aminosalicylic acids.

The present paper reports the synthesis of some *N*-(diethylaminoethyl)-4-substituted aminobenzoate quaternary salts, *N*-(diethylaminoethyl)-4-substituted aminobenzamide quaternary salts, 4-substituted acylaminobenzamide quaternary salts, and 4-substituted acylaminosalicylamide derivatives and their evaluation for antispasmodic and/or cardiovascular effects.

RESULTS AND DISCUSSION

Synthesis—The key intermediates, 4-substituted acylaminobenzoyl chlorides (I*a*–I*d*, Table I), were prepared by the classical method using thionyl chloride and the appropriate acid. *N*-(Diethylaminoethyl)-4-substituted aminobenzoate derivatives (III*a*–III*o*, Scheme I and Table II) were prepared by: Method A, condensation of the appropriate acid



Scheme I

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