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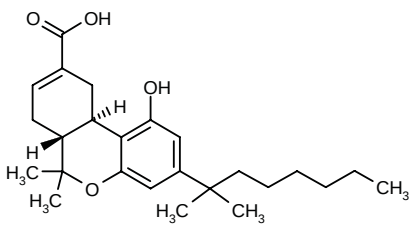
Ajulemic Acid

Antiinflammatory
Oncolytic

CT-3

1',1'-Dimethylheptyl-Δ^8-tetrahydrocannabinol-11-oic acid

(6a*R*,10a*R*)-3-(1,1-Dimethylheptyl)-1-hydroxy-6,6-dimethyl-6a,7,10,10a-tetrahydro-6*H*-dibenzo[*b*,*d*]pyran-9-carboxylic acid



$C_{25}H_{36}O_4$ Mol wt: 400.5554

CAS: 137945-48-3

EN: 236795

Synthesis

The aromatic portion of the molecule is generated by the condensation of 1,6-dimethoxyphenol with 1,1-dimethylheptanol in the presence of methanesulfonic acid at 50 °C. The crude product is then esterified with diethyl phosphite and triethylamine with cooling to yield the diethyl phosphate derivative. Reduction with lithium in liquid ammonia produces 1-(1',1'-dimethylheptyl)-3,5-dihydroxybenzene (I) which is processed crude to the next step. This involves the condensation of the p-menthenediol (II) with the dimethylheptyl resorcinol (I) catalyzed by *p*-toluenesulfonic acid to give the dimethylheptyl analog of Δ^8-THC (III). Following acetylation of the phenolic group, the allylic methyl group is oxidized to the aldehyde (IV) using selenium dioxide. Further oxidation to the carboxylic acid is accomplished by the use of sodium chlorite. Finally, ajulemic acid is obtained by removal of the acetyl group with sodium carbonate in aqueous methanol. Scheme 1.

Description

White crystalline solid, m.p. 96-9 °C, soluble in most organic solvents except hexane. Soluble in aqueous buffers above pH 8.

Introduction

Considerable effort has been expended both in academic and industrial laboratories to design a cannabinoid derived drug that would have a low potential for abuse. The efforts were spurred in large measure by the long history of the use of cannabis preparations for the treatment of pain, inflammation and a host of other medical problems. Literally hundreds of compounds have been synthesized and tested, however, few have reached the stage of human testing and only one, nabilone, is currently used in limited applications. THC, the principle psychoactive component of cannabis, is available as an oral medication (*Marinol*) for use as an antiemetic and as an appetite stimulant for AIDS patients. However, its potential for abuse has discouraged its acceptance and use by physicians for a wider range of therapeutic applications.

Ajulemic acid (CT-3) is currently being developed to achieve the goal of providing a cannabis-derived synthetic molecule that will have a low potential for abuse (1, 2). The rationale used in designing the structure of ajulemic acid was based on several reports in the scientific literature relating to the metabolic transformations of THC. As early as 1972, it was demonstrated that the principle metabolic route for THC involved its stepwise oxidation to

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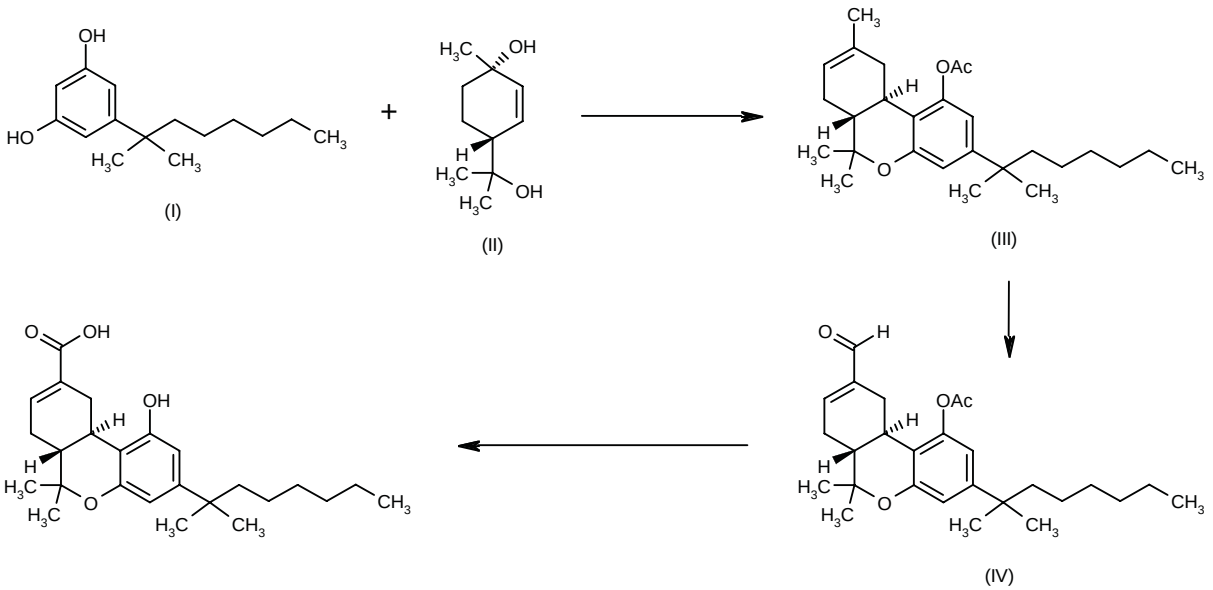
Scheme 1: Synthesis of Ajulemic Acid



a series of carboxylic acid derivatives (3). In contrast to THC, the acids showed little activity in several studies of psychotropic responses in both animals and humans (4). This led to the perception that the acid metabolites are "inactive" and discouraged further studies of their possible pharmacological properties.

The above situation was altered when a series of reports showed that the acids, in fact, possess biological actions that could be exploited for therapeutic applications (5-9). In addition, it is generally accepted that cannabinoids as a class are relatively safe drugs, especially when compared with analgesics such as the opiates and their synthetic derivatives. A disadvantage of the acid metabolites was their low potency in preclinical testing; however, this problem has been successfully resolved with the discovery of ajulemic acid (10). The recent completion of a phase I safety trial confirmed ajulemic acid's low abuse potential over the expected range of therapeutic doses (Atlantic, unpublished data).

Pharmacological Actions

A well-defined mechanism of action for ajulemic acid has not yet been advanced; however, several observations allow some tentative conclusions to be made. Modest binding to the known cannabinoid receptors, CB1 and CB2 (11, 12), has been reported and inhibition of action by receptor-specific antagonists as well as stereospecificity (10, 13) has been observed, suggesting a possible role for these receptors in the actions of ajulemic acid. However, these data pose something of a dilemma when considered together with the fact that it does not produce psychoactivity (Atlantic, unpublished data), a process that is believed to require the activation of CB1. One possible explanation is that ajulemic acid, in addition to activating the receptor, also selectively inhibits a downstream event required for psychoactivity but not needed for other actions. The lack of information concerning the molecular events leading to cannabinoid-induced psychoactivity in general precludes further speculations on this point.

Data have been obtained on several biochemical effects that may have relevance for the antiinflammatory properties of ajulemic acid. Cannabinoid receptor binding in intact cell models causes the release of free arachidonic acid, suggesting the activation of one or more phospholipases (14). Recent data show that ajulemic acid can likewise stimulate the release of arachidonic acid in similar models (Fig. 1). Ajulemic acid also inhibits COX-2 (15) and 5-lipoxygenase (D. Morgan, unpublished data) but not COX-1 (15). The weak COX-1 inhibitory activity is in agreement with its lack of ulcerogenicity (16) and its inability to prevent platelet aggregation (17). Effects on specific cytokine levels and on the activation of NF-kappaB have also been observed (R. Zurier, unpublished data).

Ajulemic acid has been subjected to a variety of preclinical *in vivo* models for antiinflammatory activity where it showed impressive potency (10, 15). For example, orally administered ajulemic acid reduced the induction of paw edema in mice injected with arachidonic acid with an ED_{50} of 0.02 mg/kg p.o. (Fig. 2). Similar results were obtained when edema was induced by platelet actvating factor, where an ED_{50} of 0.05 mg/kg p.o. was observed (10). Carrageenan-induced edema was also inhibited at a

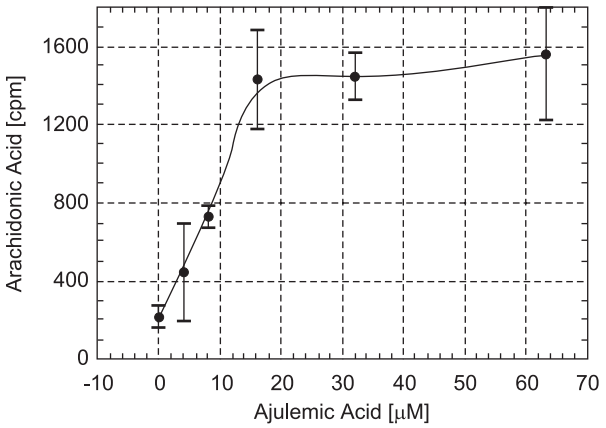


Fig. 1. Ajulemic acid stimulates the release of arachidonic acid from C-6 glioma cells in culture. Cells at 90% confluency in 24 well dishes were labeled with tritiated arachidonic acid for 4 h. Following two washings with RPMI medium containing 0.1% BSA, the cells were treated with ajulemic acid in DMSO for 60 min at concentrations ranging from 0-63 μM. Aliquots of the media were sampled for determination of radioactivity which consisted of >95% arachidonic acid. The values shown are the means of three wells ± S.D.

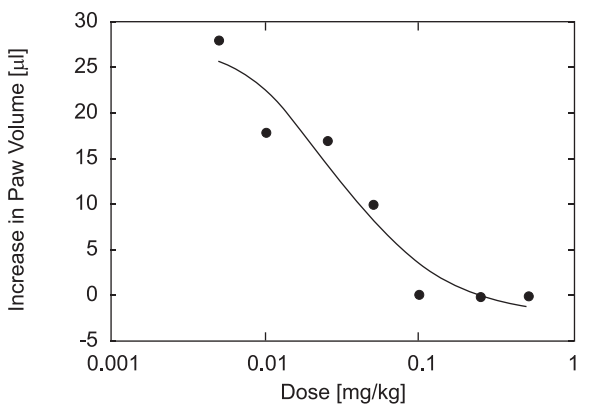


Fig. 2. Inhibition of arachidonic acid-induced paw edema in mice (n = 5) by ajulemic acid. The data shown are obtained using the conditions described in ref. 10. Briefly, arachidonic acid (1.0 mg in 25 μl of saline) was injected s.c. into the hind paw 30 min after the drug in 50 μl of peanut oil given p.o. The paw volumes were measured after an additional 30 min. Data were analyzed by ANOVA for significance (p = 0.006).

higher dose, showing an ED_{50} of 2.2 mg/kg i.v. (Roche Biol. Res., unpublished data). Zurier *et al.* have reported that the migration of leukocytes into a subcutaneous air pouch following injection of TNFα and IL-1β was markedly reduced at doses of 0.1 and 0.2 mg/kg p.o. (15). Finally, ajulemic acid at a dose of 0.2 mg/kg p.o. reduced several of the effects related to inflammation in an adjuvant-induced arthritis model in rats (15). Histological examination of randomly selected specimens from this study revealed a remarkable joint sparing effect in ajulemic acid-treated animals compared with vehicle-treated controls (20% *vs.* 80% ankylosis).

Analgesic activity in a variety of animal models has been reported by several independent groups. Burstein *et al.* (10, 17) observed antinociceptive action for ajulemic acid in the mouse hot plate assay at 55 °C. This was confirmed by Dajani *et al.* (16) who reported an ED_{50} of 6.7 mg/kg i.g. which was equipotent with morphine in their study. Of interest was the somewhat longer duration of activity for ajulemic acid compared with morphine. The same group also reported a study using the tail clip assay in which an ED_{50} of 4.4 mg/kg was obtained. In the PPQ writhing assay, Burstein *et al.* (17) found activity for ajulemic acid with an ED_{50} of 1.24 mg/kg. In the same publication, they also reported inhibition in both the first and second phases of the mouse formalin test, suggesting the possibility of both centrally and peripherally mediated analgesia.

Allodynia induced by paw injection of PAF in rats was shown by Walker et al. to be completely reversed following the administration of 5 mg/kg of ajulemic acid (18). Higher doses resulted in analgesia as measured by increased tolerance to mechanical pressure. No effect on motor function (rotarod assay) was seen under the conditions of the assay. In a similar model, analgesia was observed following paw injection of either carrageenan or complete Freund's adjuvant (Roche Biol. Inst., unpublished data). The ED_{50} values for ajulemic acid were 1.8 and 1.1 mg/kg, respectively. In another study, at a single dose of 30 mg/kg, ajulemic acid failed to produce analgesia in the hot plate and writhing assays, suggesting the occurrence of a bell-shaped, dose-response relationship (J&J PRI, unpublished data).

Recently, Recht *et al.* have shown that ajulemic acid is highly effective in inhibiting the proliferation of cancer cells (13). The effect on normal cells was much less and, in all cases, cell growth resumed upon withdrawal of ajulemic acid. Changes in lipid metabolism and prostaglandin synthesis occurred concurrently, raising the possibility of the involvement of PPARγ. The same group also demonstrated a modest but significant *in vivo* antitumor effect in a subcutaneous mouse model at a dose of 0.2 mg/kg p.o. administered thrice weekly.

Toxicity

The most significant unwanted side effect of many antiinflammatory agents is the formation of gastrointestinal ulcers. For this reason, ajulemic acid was carefully examined (16) to detect any possible ulcerogenicity. When given acutely to rats in doses up to 1000 mg/kg, no evidence for ulcer formation was seen. Chronic i.g. administration of up to 30 mg/kg likewise resulted in no ulcerogenicity, whereas the indomethacin control rats showed extensive ulcer formation.

Because of its relationship to THC and its potent analgesic properties, ajulemic acid was studied for possible induction of opiate-like physical dependence in a 14-day

rat study. None of the typical opiate withdrawal effects such as writhing, diarrhea, wet dog shakes, etc. were observed (Atlantic, unpublished data), indicating that ajulemic acid has a low dependence liability. There were no effects on renal, cardiovascular or gastrointestinal function and no signs of respiratory depression as well. Lethal doses were estimated following single doses in mice (600 mg/kg) and in rats (400 mg/kg). Ajulemic acid was well tolerated in a 14-day study at doses up to 50 mg/kg. Three different standard tests for mutagenic potential gave negative findings.

Human Pharmacokinetic and Safety Studies

An FDA approved phase I, single-center, double-blind, randomized, placebo-controlled study of ajulemic acid has recently been completed (Atlantic, unpublished data). The purpose of the study was to determine the safety, tolerability and pharmacokinetics of a single oral dose of ajulemic acid in healthy adult male volunteers. A total of 32 subjects were given doses ranging from 0-10 mg and monitored for 24 h following treatment. Data from clinical laboratory tests, cardiovascular measurements and tests for psychoactivity were obtained. The latter consisted of a 12 item yes/no questionnaire developed by the Addition Research Center at The National Institute on Drug Abuse and is commonly referred to as the ARCI-M scale. It is designed to detect the full range of subjective responses experienced by marihuana users and has been validated by subjects following marihuana smoking. The subjects in the ajulemic acid study were told that they would be receiving a synthetic derivative of THC.

The data from all dose levels showed that ajulemic acid is safe and well tolerated. The scores obtained with the ARCI-M test showed no significant differences between placebo- and ajulemic acid-treated volunteers. This finding is in agreement with the reported preclinical studies in rodents (6, 10) and supports the conclusion that ajulemic acid does not produce a marahuana-like “high” at doses within the expected therapeutic range. Pharmacokinetic measurements using mass spectrometry revealed that ajulemic acid is rapidly absorbed following oral administration and is eliminated with a terminal half-life of around 3 h. The AUC and C_{max} values showed a linear relationship when compared over the dose range of 1-10 mg/subject.

Sources

Discovered by Sumner Burstein, University of Massachusetts Medical School; licensed to Atlantic Technology Ventures, Inc., New York (US).

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