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3-(15-Hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen-1-one and its Effect on Neuropeptide Secretion

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Abstract—The aim of the present study was to describe the synthesis of a trimethyl cyclohexenonic long chain fatty alcohol (*t*-CFA), and analyze its biological activity. Specifically, 3-(15-hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen-1-one, the *t*-CFA containing 15 carbon atoms on the side chain (*t*-CFA $n=15$) stimulated arginine vasopressin secretion in nerve terminals of the neurohypophysis. This effect was inhibited by extracellular calcium depletion, which suggets that *t*-CFA $n=15$ stimulates neuropeptide secretion through a calcium-dependent exocytosis mechanism. © 2000 Published by Elsevier Science Ltd.

Introduction

Since the demonstration of in vitro neurotrophic activity of *n*-hexacosanol, a long chain primary alcohol containing 26 carbon atoms,[1] we have focused our attention on long chain fatty alcohols that contain a functionalized nucleus in order to improve their physicochemical and bioavailability properties. Our aim is the search of substances which are able to promote the development of the nervous system, and in particular the establishment of neuronal networks.

Previous studies showed that monomethyl and dimethyl cyclohexenonic long chain fatty alcohols with an appropriate length of the side chain ($n=14$ carbon atoms for the most active in these series) are potent inducers of neurite outgrowth.[2] In the present report, we show that 3-(15-hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen-1-one, a trimethyl cyclohexenonic long chain fatty alcohol containing 15 carbon atoms on the side chain (*t*-CFA $n=15$, **1**), with neuritogenic activity,[3] is a potent inducer of arginine vasopressin secretion in nerve terminals of the neurohypophysis, a well-characterized example of neuropeptide exocytosis.[4] Although the cellular mechanism underlying this effect is still unclear, the structural homology of these compounds with endogenous long chain fatty alcohols, which are involved in ether lipid biosynthesis,[5] allows us to hypothesize that *t*-CFA $n=15$ could act at the membrane level.

Chemistry

The compound 3-(15-hydroxypentadecyl)-2,4,4-trimethyl-2-cyclohexen-1-one **1** was synthesized in seven steps from commercially available geranyl bromide **2**.

In an methanol medium, **2** reacted with benzenesulfinic acid sodium salt to give the corresponding geranylphenylsulfone **3**, which was cyclized as described by Krishna et al.[6] and Torri et al.[7] to afford a mixture of **4** and **5** (85:15) in 90% yield (Scheme 1). The two isomers were separated by successive recrystallizations in hexane, and the desired **4** was obtained in 74% yield with a purity over 99%.

As shown in Scheme 2, sulfone **4** was coupled with the unprotected 14-bromo-tetradecan-1-ol in a one-pot procedure using *n*-butyllithium in the presence of HMPA to give **6**. After reductive desulfonation by means of 6% Na/Hg amalgam,[8] the resulting alcohol **7** was protected by an acetate to give **8** which was oxidized to the corresponding α,β-unsaturated ketone **9** using $RuCl_3$ as catalyst (0.7% mol) and 70% *t*BuOOH.[9] The deprotection of the alcohol by K_2CO_3 in methanol gave **1** in 91% yield.[10]

In the same manner, compounds with different side chain lengths (12 to 18 carbon atoms) were synthesized, reactivities and yields being similar to those of **1**.

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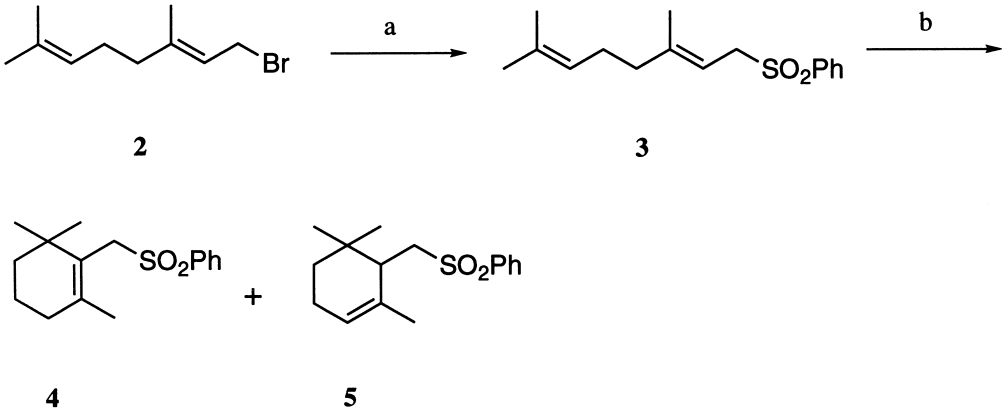
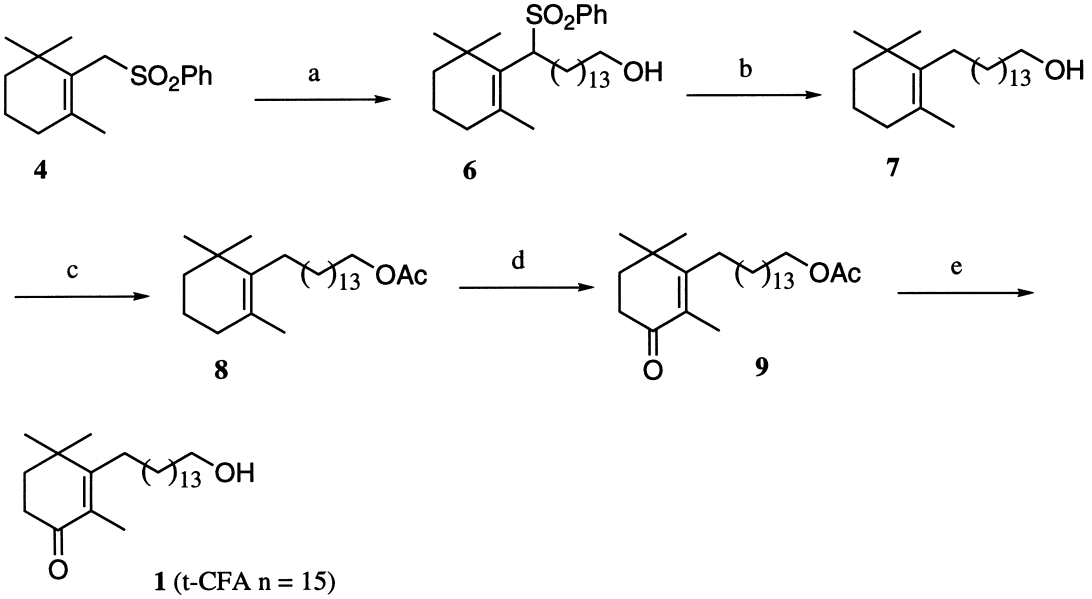
Biological Activity

In order to determine whether *t*-CFA $n=15$ exhibits any specific activity in neuropeptide secretion, mouse neurointermediate lobes (NILs) were incubated in a static culture system to quantify arginine vasopressine (AVP) release. Briefly, pituitary glands from male FVB/N mice were rapidly dissected, and further separated into anterior lobe and NIL under a microscope. NILs were preincubated for 1 h at 37 °C in Krebs–Ringer bicarbonate buffer (KRB, pH 7.4) containing (in mM): NaCl 118.5, KCl 4.75, $CaCl_2{\cdot}2H_2O$ 2.5, KH_2PO_4 1.2, $MgSO_4{\cdot}2H_2O$ 1.2, and supplemented with 0.2% glucose and 0.1% bovine serum albumin. Tissues were then treated with 500 nM *t*-CFA $n=15$ in both normal and calcium-free medium (KRB without $CaCl_2$, and supplemented with 2.5 mM $MgCl_2$, and 1 mM EGTA). After 20 min, medium samples were collected, and stored at −20 °C until being radioimmunoassayed for AVP as previously described.[11] *t*-CFA $n=15$ was dissolved in ethanol and diluted to render a final concentration of 0.05% ethanol. All NILs were incubated in the same conditions.

As illustrated in Figure 1, *t*-CFA $n=15$ induced a significant increase (5.09-fold) in AVP secretion. To ascertain whether this stimulatory effect was due to activation of a regulated exocytotic pathway rather than non-specific release, NILs were treated with *t*-CFA $n=15$ in the absence of extracellular calcium. Interestingly, AVP secretion was completely abolished by calcium depletion. The crucial role of calcium in neuropeptide exocytosis, and the stimulation of AVP secretion through calcium-dependent mechanisms have been largely demonstrated.[12] Our present findings showing a calcium-dependent stimulatory effect suggest that *t*-CFA $n=15$ could trigger an influx of Ca^{2+} ions from the extracellular medium, which in turn would facilitate neuropeptide secretion.



Scheme 1. Reagents: (a) $PhSO_2Na$, MeOH, 0 °C, 1 h (80%); (b) H_2SO_4 32 equiv, AcOH, 12 °C, 30 min (90%).



Scheme 2. Reagents: (a) (1) *n*-BuLi, THF, HMPA, $CHPh_3$, −78 °C to rt, 1 h; (2) $Br(CH_2)_{14}OH$, THF, −78 °C to −20 °C, 2 h (87%); (b) Na/Hg 6%, MeOH, Na_2HPO_4, 0 °C to rt, 3 h (90%); (c) Ac_2O, pyridine, rt, 1 h (98%); (d) $RuCl_3$ 0.7% mol, *t*BuOOH 70%, cyclohexane, H_2O, rt, 6 h (53%); (e) K_2CO_3, MeOH, H_2O, rt, 2 h (91%).

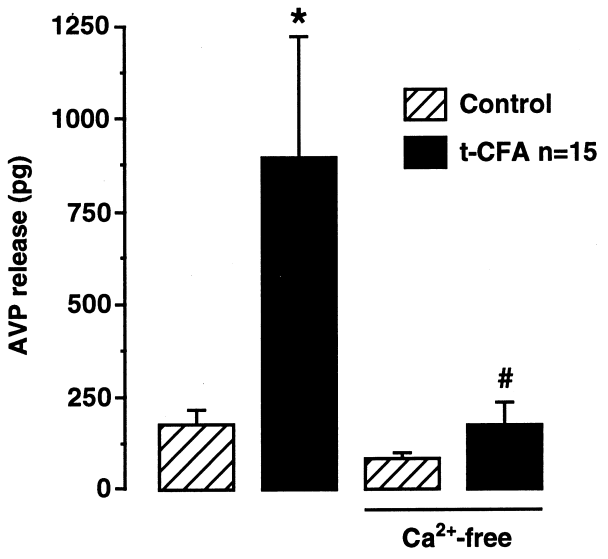


Figure 1. Effect of *t*-CFA $n=15$ on AVP secretion. NILs were treated with 500 nM *t*-CFA $n=15$ in the presence or absence of Ca^{2+} in the culture medium. The data represent the mean±SE ($n=4$), and they are expressed as total pg/300 mL. $*P<0.05$ vs control, $\#P<0.05$ vs *t*-CFA $n=15$, determined by one-way ANOVA, followed by Student–Newman–Keuls test.

The neurohypophysis is a key organ implicated, through release of AVP and oxytocin, in numerous physiological functions, including osmotic homeostasis, pregnancy, and lactation.[13] Thus, the utilization of trimethyl-CFAs as potent neural lobe secretagogues opens new lines of investigation in biomedical research.

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