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Progress of an anti-convulsant drug from discovery to manufacture

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Abstract

Inhibition of GABA uptake is a useful strategy in the design of anti-convulsants. The development of an anti-epileptic drug from discovery to process development and manufacture is described. Synthesis of the human metabolite and tablet degradates has been achieved.

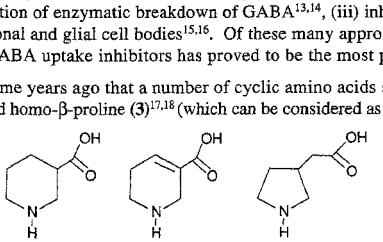
Key words: GABA uptake, anti-convulsants, anti-epileptic, synthesis, metabolite and degradates.

1. Introduction

Epilepsy is a disorder characterized by recurrent spontaneous electrical discharges within the brain which are manifested by clinical seizures. Four million patients in the USA are afflicted with this ailment. Current targets for therapeutic intervention include blocking of receptors of excitatory amino acids, modulating excitatory membrane ion channels and enhancing the neurotransmittory effect of γ-aminobutyric acid (GABA)[1,2].

Gamma-aminobutyric acid is the principal inhibitory neurotransmitter in the mammalian central nervous system[3]. Dysfunctioning of GABA-ergic synapses has been invoked for Parkinson's disease[4–6], epilepsy[7,8] and some forms of schizophrenia[9,10]. The approaches to the enhancement of GABA-ergic function in humans comprise: (i) direct agonism of GABA receptors[11,12], (ii) inhibition of enzymatic breakdown of GABA[13,14], (iii) inhibition of uptake of GABA into the neuronal and glial cell bodies[15,16]. Of these many approaches, the design of substrates that are GABA uptake inhibitors has proved to be the most promising.

It was discovered some years ago that a number of cyclic amino acids such as nipecotic acid (**1**), guvacine (**2**), and homo-β-proline (**3**)[17,18] (which can be considered as conformationally



1 **2** **3**

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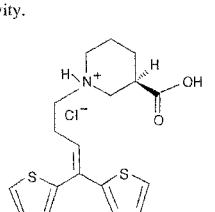
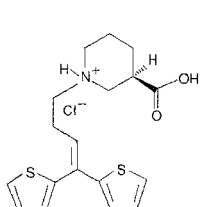
restricted GABA analogs[19]) display *in vitro* activity as inhibitors of [^{3}H]-GABA uptake. However, detailed investigation revealed that these cyclic amino acids do not readily cross the blood brain barrier[17,20,21]. Preparing more lipophilic prodrug esters[11] of **(1)** and **(2)** provided compounds which were protective in various seizure models[17,22]; despite this, their cholinergic effects[23] had a negative influence on their *in vivo* utility.

An extensive program to synthesize a series of novel and selective GABA uptake inhibitors was undertaken[24]. One compound from the series, (R)-1-[4,4-*bis*-(3-methyl-2-thienyl)-3-butenyl]-3-piperidinecarboxylic acid (Tiagabine) **(I)** has progressed to phase III human clinical trials for treatment of epilepsy. The pharmacology[25,26] in laboratory animals and biochemistry[27,28] provide evidence of the compound's potency and selective mode of action. Tiagabine also possesses some analgesic[29] and anxiolytic[30] activity. The drug has suitable lipophilicity for availability in the central nervous system. It thus represents a prototype for future drug development in this field. The results of human clinical trials of Tiagabine have been reported[31].

Desmethyltiagabine **(II)** is an example of an unsymmetrical analog that shows interesting biological activity.



I

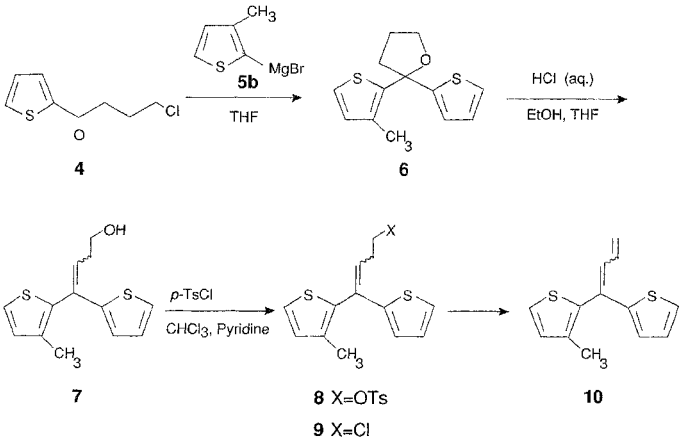


II

2. Synthesis of unsymmetrical analogs (desmethyltiagabine) (II)[32]

Retrosynthetic analysis of the target molecule would suggest a strategy of alkylating a nipecotic acid residue (protected as the ester derivative) with a 4-halo- or 4-tosyl-1,1-diaryl-1-butene. Such an approach is shown in Scheme 1.

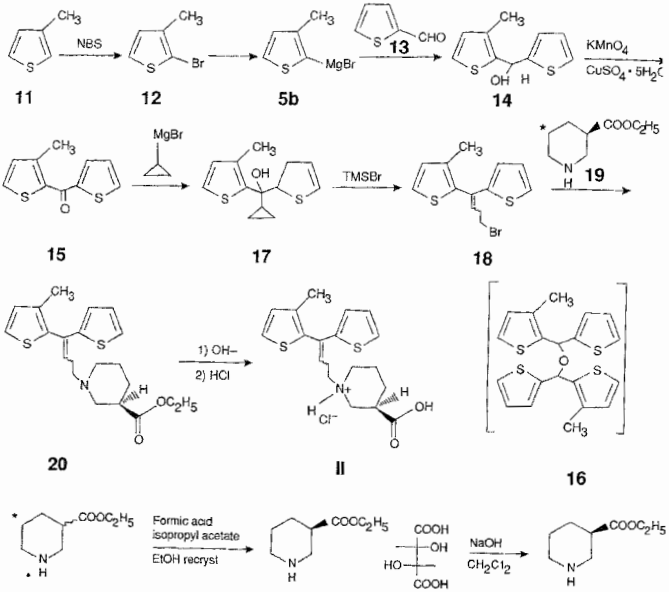
Reaction of 4-chloro-1-(2-thienyl)-1-butanone **(4)** with the Grignard reagent **(5b)** provided the 2,2-disubstituted tetrahydrofuran **(6)** in good yield. The tetrahydrofuran ring could be opened with concomitant dehydration to provide the unsymmetrical butenol **(7)**. Conversion to the *p*-toluene sulfonate derivative **(8)** with *p*-toluenesulfonyl chloride in pyridine/chloroform at 45°C was a facile process; at reflux temperature, the corresponding chloride derivative **(9)** was obtained. These derivatives could be used to alkylate nipecotic acid esters but the yields were variable. The chloride displacement in particular was not amenable to catalysis by crown ethers, DMAP, etc. Elimination to the diene **(10)** was also a frequent problem.



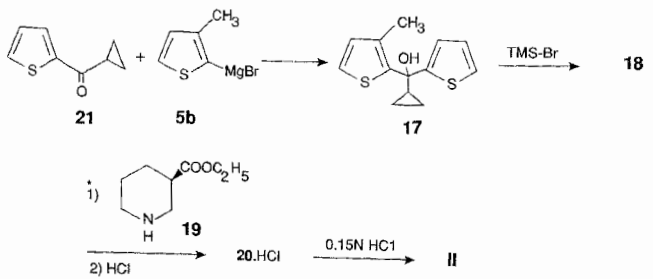
SCHEME 1.

We now report technically superior syntheses that were amenable to scale up. Scheme 2 depicts the first of two methods that appeared to be particularly successful.

3-Methylthiophene (**11**) was brominated with N-bromosuccinimide by standard procedures[33] to yield 2-bromo-3-methylthiophene (**12**). No product arising from the bromination of the methyl group was discernible. Grignard reagent (**5b**) was prepared in high yield[33] and condensed with 2-formylthiophene (**13**) to yield the carbinol (**14**). This carbinol is unstable to storage and is readily converted to the corresponding ether dimer (**16**) after standing even at $-10^{\circ}C$ or on contact with acids. The ether dimer (**16**) is a crystalline compound; its structure was confirmed by nmr and mass spectra. The Grignard reagent was superior to the aryl lithium reagent; with the latter, more isomeric products were obtained. Carbinol (**14**) was oxidized with a mixture of potassium permanganate and copper sulfate pentahydrate to the ketone (**15**)[34]. The reaction was slow and necessitated the addition of fresh portions of the reagent after 24 h periods. A small amount of (**16**) was formed in this reaction. Oxidation with numerous other reagents such as MnO_2, pyridinium chlorochromate on alumina, or oxidation under phase transfer conditions yielded the ketone in much lower yields which were difficult to reproduce. It is useful to note that the ketone (**15**) could not be directly prepared by reaction of the Grignard reagent (**5b**) with 2-cyanothiophene or the acid chloride of thiophene-2-carboxylic acid[35]. The carbonyl carbon of the dithienyl ketone (**15**) was refractory to attack by Grignard reagents designed to introduce the straight chain 3-carbon fragment. Reaction with cyclopropyl magnesium bromide furnished the carbinol (**17**) in high yields[36]. The cyclopropyl ring was opened with simultaneous dehydration and bromination with hydrobromic acid in acetic acid or with bromotrimethylsilane to yield the 4-bromo-1,1-diaryl-



SCHEME 2 (Method A).



SCHEME 3 (Method B).

1-butene (**18**)[37]. Ethyl (R,S)-piperidine-3-carboxylate was resolved by literature procedures[38] to furnish the R-enantiomer as the L-(+)-tartrate. Conversion to the free base (**19**) was followed by condensation with (**18**) in acetone to yield (**20**). Hydrolysis of the ester residue with base followed by extraction and acidic workup yielded the hydrochloride salt of desmethyltiagabine(**II**). It is important to note the crystallization of the product as a methylene chloride solvate. The material revealed a slow loss of methylene chloride on standing, and was shown to be a 90:10 mixture of the Z and E isomers, respectively, by nmr.

Further improvements in the synthesis are shown in Scheme 3 (Method B). Cyclopropyl thien-2-yl ketone (**21**), available commercially, was subjected to a Grignard reaction with (**5b**) to yield the carbinol (**17**) in excellent yield. Bromotrimethylsilane-mediated opening of the cyclopropyl ring led to the 4-bromo derivative (**18**). Condensation with ethyl R-(–)-piperidine-3-carboxylate (**19**) was conducted in isopropyl acetate as solvent with anhydrous lithium carbonate as the base. The transformation was cleaner and did not provide any of the diene (**10**) arising from elimination of hydrogen bromide. The alkylation of the nipecotate residue could also be directly effected with a mixture of tartrate salt of (**19**), lithium carbonate and isopropyl acetate. The yields were, however, marginally lower. Compound (**20**) was readily isolated as the hydrochloride; isopropyl acetate was superior to all other solvents used in this reaction.

Acid-catalyzed hydrolysis with aqueous hydrochloric acid was a facile process; the hydrochloride salt of the product could be crystallized out of the same solution. 0.15N Hydrochloric acid was found to be optimally effective in cleaving the ester without racemizing the chiral center. In conclusion, the methods developed here are general ones that can be adapted to the synthesis of several analogs of Tiagabine.

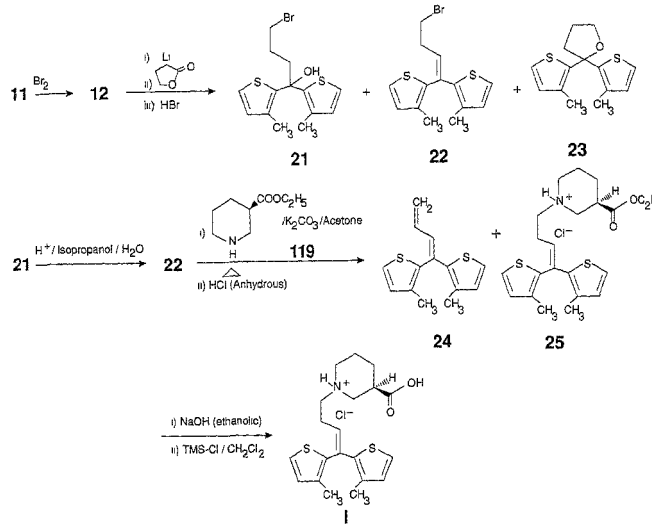
3. Synthesis of symmetrical analogs (Tiagabine)(I)

Schemes 4 and 5 describe the synthesis of these compounds.

The symmetrical structure makes the synthesis more facile than the unsymmetrical congener. The intricate details of each synthetic transformation are at present a trade secret. Considerable work has been done in our laboratories to optimize the reactions and generate the product in optimum yield and purity.

4. Human metabolite of Tiagabine (5-hydroxytiagabine) (38)[39]

After oral or intravenous administration of ^{14}C-Tiagabine (30 mg/kg) to rats, the metabolic profile in urine was characterized by two major peaks, which together accounted for about 90% of the urinary radioactivity or 10–15% of the ^{14}C-dose. Upon isolation, each peak was shown to convert to the other and eventually equilibrate to an approximate 1:1 mixture showed identical protonated molecular ions (*m/z*=392) and daughter ion fragmentation patterns suggested thiophene ring oxidation (+16 amu). The ^{1}HNMR spectrum of an equilibrium mixture of the two metabolites revealed a pair of thiophene ring proton singlets (δ 6.42 and 6.44) and the absence of the olefinic proton present in the Tiagabine spectrum (δ 5.98). Based on these data and literature precedents for the existence of hydroxythiophenes as keto tautomers, it is presumed that oxidation in one of the thiophene rings of Tiagabine (**I**) formed 5-hydroxytiagabine (**38**). This was believed to be present entirely as E and Z isomers of the tautomeric 5-oxo form. The urinary metabolites were also identified as human metabolites



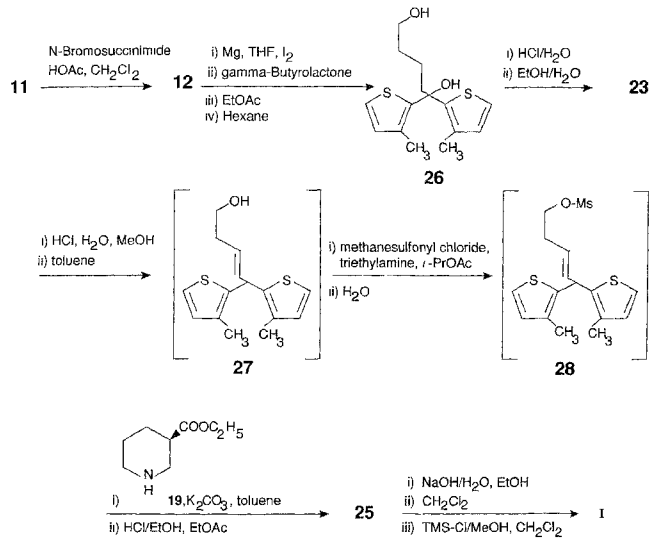
SCHEME 4 (Method A).

following oral administration of ^{14}C-Tiagabine to adult male subjects. To ensure the position of oxidation in Tiagabine, a total synthesis starting from well-characterized starting materials was performed. Two approaches for the preparation of 5-hydroxytiagabine (**38**) shown in Schemes 6 and 7 have been studied.

(**11**) is brominated with N-bromosuccinimide by standard procedures[40] to 2-bromo-3-methylthiophene (**12**) which then is chlorinated to give 2-bromo-5-chloro-3-methylthiophene (**29**).

Initial attempts to selectively chlorinate (**12**) at the 5 position failed. Chlorination of (**12**) with N-chlorosuccinimide[41] or sulfuryl chloride[42] has been reported to give (**29**) in 62–90% yield. In our hands, these procedures gave mixtures of (**29**), 2,5-dichloro-3-methylthiophene and 2,5-dibromo-3-methylthiophene in the ratio 4.7:1:1 according to gc/ms analysis. The two side products probably formed by halogen exchange can also be observed in high-field ^{1}H NMR spectra. A similar mixture was obtained using chlorine in dichloromethane in the presence of mercuryoxide[43].

2-Bromo-5-chloro-3-methylthiophene (**29**) was converted to 5-chloro-3-methylthien-2-ylmagnesium bromide (**30**) which was added to 2-formyl-3-methylthiophene(**31**) producing the *bis*(thien-2-yl)methanol (**32**). 2-Formyl-3-methylthiophene (**31**) in its turn was prepared by formylation of 3-methylthien-2-ylmagnesium bromide (**30**) with N,N-dimethylformamide

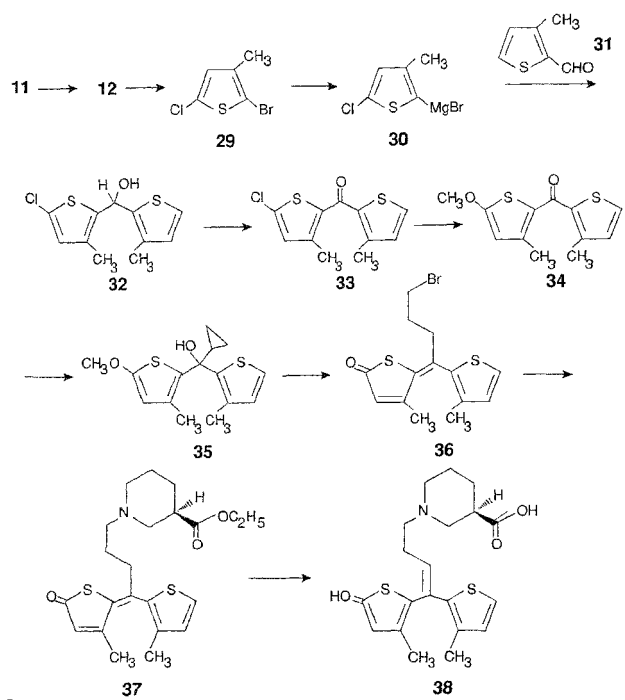


SCHEME 5 (Method B).

using known methodology. *Bis*(thien-2-yl)methanol (**32**) is sensitive to acid but could be oxidized to the corresponding ketone (**33**) under controlled conditions. Manganese dioxide and pyridinium chlorochromate supported on basic aluminium oxide were used, the latter providing the best yield.

The chlorine in *bis*(thien-2-yl)ketone (**33**) was replaced with a methoxy group to give compound (**34**) by treatment with sodium methoxide in N,N-dimethylformamide–methanol(9:1). These conditions seem particularly effective in substitution of weakly reactive chlorine with methoxide ions. In the present case, sodium methoxide in dimethylformamide in the absence of methanol gave slow conversion to (**34**) and formation of several unidentified byproducts. While displacement of the chlorine in compound (**33**) could be achieved by this procedure corresponding displacement of bromine and iodine only afforded dehalogenation.

5-Methoxyketone (**34**) was treated with cyclopropylmagnesium bromide to give cyclopropyl methanol (**35**). Treatment of this compound with bromotrimethylsilane resulted both in demethylation, in opening of the cyclopropyl ring with elimination of trimethylsilanoxide, and in migration of the double bond thus formed to give the conjugated system (**36**). The aliphatic bromine atom of compound (**36**) was displaced with ethyl (R)-3-piperidinecarboxylate and the resulting ester (**37**) was hydrolyzed in a standard fashion with production of 5-



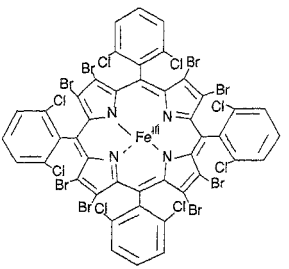
SCHEME 6.

hydroxytiagabine (**38**). Acidic hydrolysis was found superior to basic providing the acid (**38**) in virtually quantitative yield.

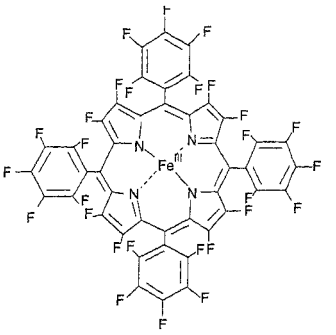
5. Biomimetic synthesis of 5-hydroxytiagabine(38)[39]

The synthetic problems associated with the method described above prompted us to look for a novel method of preparation. 5-Hydroxytiagabine is formed *in vivo* by a regiospecific cytochrome P-450 mono-oxygenase-mediated hydroxylation; extensive work has established the intermediacy of high-valent iron porphyrin intermediates in similar transformations[44]. Sterically protected and electronically activated metalloporphyrins have been studied as synthetic models for cytochrome P-450-mediated epoxidations and hydroxylations. These catalysts are robust, not destroyed under strongly oxidizing conditions, and effect catalytic oxidations with high turnover numbers[44–51].

The central double bond in Tiagabine is hindered and relatively inert to epoxidation under a wide variety of reaction conditions. Treatment with hydrogen peroxide, sodium hypochlorite or *m*-chloroperbenzoic acid did not lead to significant amounts of epoxide formation. Porphyrin-assisted oxidation was therefore anticipated to be directed towards hydroxylation of the thiophene ring. This prediction was confirmed by experiment. We found octabromo tetrakis(2,6-dichlorophenyl)porphyrin Fe(III)Cl [abbreviated as $Cl_8Br_8Fe(III)TPP$] (**III**) and octafluoro tetrakis (pentafluoro phenyl) porphyrin Fe(III)Cl [abbreviated as 'perfluoro Fe(III)TPP'] (**IV**) to be very effective in achieving hydroxylation of the thiophene ring.



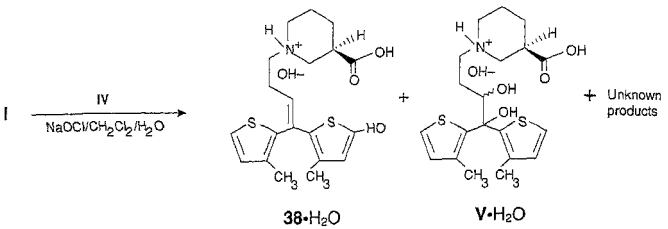
III



IV

The hemins were synthesized by the methods of Traylor, Dolphin and Tsuchiya[51-53]. Condensation of the appropriate aldehydes (2,6-dichlorobenzaldehyde and pentafluorobenz- aldehyde, respectively) with pyrrole in the presence of anhydrous zinc chloride in refluxing lutidine yielded the zinc complexes of the porphyrins after chromatographic purification. Halogenation of the pyrrole moiety with N-bromosuccinimide in refluxing CCl_4 or with anhydrous Ag(I)F in refluxing CH_2Cl_2 furnished the perhalogenated materials. Demetallation with trifluoroacetic acid followed by conversion to the hemin (Fe^{II} Cl_2/DMF at reflux) by the method of Adler[54] and Kobayashi[55] provided the hemins after alumina chromatography.

Treatment of Tiagabine (**I**) with the synthesized hemins in a methylene chloride/water biphasic system with NaOCl or 30% H_2O_2 as the exogenous oxygen donor gave 5-hydroxytiagabine. H_2O(**38**.H_2O) in 62–74% yield. Phase transfer catalysts were not required. Conversion of Tiagabine to the base allowed reaction with the hemin and *t*-butyl hydroperoxide in methylene chloride. The yields were lower (40%) and more side products were discernible. The synthesized metabolite revealed complete spectroscopic and chromatographic identity with the authentic sample of the human metabolite. `Dihydroxytiagabine' (**V**) arising, presumably, *via* epoxidation of the central double bond followed by epoxide ring opening formed only in small amounts. This product was confirmed to the dihydroxy material by independently subjecting (**I**) to the Sharpless asymmetric dihydroxylation[56,57].



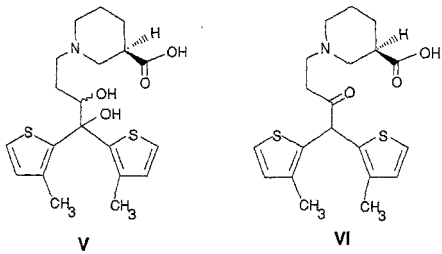
SCHEME 7.

Polar and apolar side products of the metalloporphyrin-assisted hydroxylation have not been conclusively identified. This method is amenable to large-scale synthesis of human metabolites. Experiments are under way to use the iron or manganese porphyrins in the synthesis of human metabolites of drugs currently under clinical investigation. Availability of larger amounts of metabolites would facilitate biochemical and toxicological studies.

6. Oxidative degradation products of tiagabine[57]

Initial liquid and tablet formulations of Tiagabine had a short shelf life of 1–2 years and revealed the presence of two major and other minor oxidative degradation products. The degradation products could be suppressed by the addition of anti-oxidants to the formulation. This indicated the degradation process to be of oxidative origin. The products could also be seen in stressed solutions of bulk drug exposed to ultraviolet light in the presence of air.

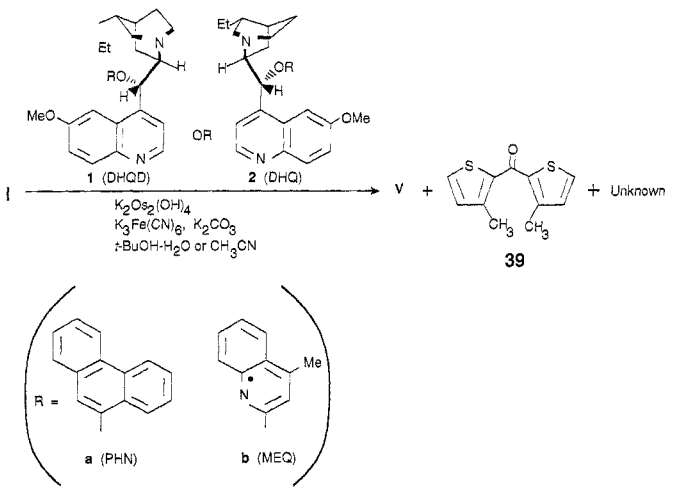
The major products were 'dihydroxytiagabine' **(V)** and 'ketotiagabine'**(VI).**



Dihydroxytiagabine (V)

A solution of Tiagabine was placed in a lightchamber and exposed to 6000 lux for 1 week. The degradation product was separated by hplc with ethanol/trifluoroacetic acid as the eluant. Neutralization of the eluates followed by desalting and freeze-drying provided the pure product.

Preparation of dihydroxytiagabine (**V**) was accomplished by the method shown in Scheme 8.



PHN 9-O-(9'-Phenanthryl)ether of dihydroquinidine
MEQ 9-O-(4'-Methyl-2'-quinolyl)ether of dihydroquinidinel

SCHEME 8.

The synthesis proved difficult in that the central double bond is hindered to be refractory to attack by reagents such as *m*-chloroperbenzoic acid, hydrogen peroxide, etc. The putative epoxide was not detected under a variety of reaction conditions; a complex mixture of products was always obtained. Reaction with osmium tetroxide/pyridine/N-methylmorpholine-N-oxide was slow and yielded the requisite diol in low yield but extraction of the product from water proved to be a problem.

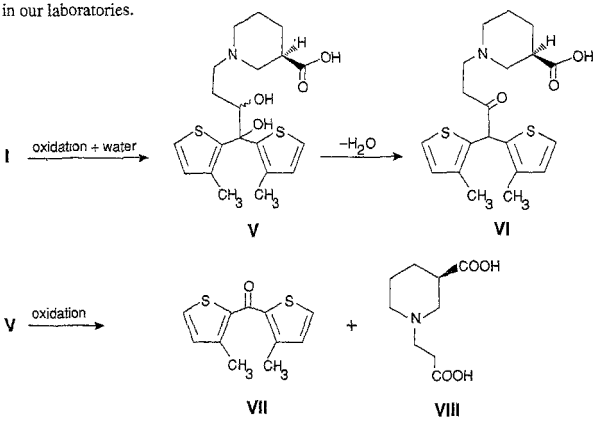
The Sharpless procedure[56] for effecting osmium-catalyzed, ligand-accelerated asymmetric dihydroxylation was successfully utilized; the reaction could also be scaled up. *Bis* (3-methylthien-2-yl)ketone (**IV**) was also a product in these reactions and was accompanied by an impurity whose structure has not been elucidated. Optimum yields of the dihydroxy material were obtained in dioxane–water/*t*-butanol–water mixtures. The use of osmium tetroxide, instead of potassium osmate, led to slower reaction and increased formation of undesired products.

The material derived from synthesis revealed complete identity with the tablet degradate; any diastereomers that formed were not resolved under our chromatographic conditions.

Attempted functionalization of the vicinal dihydroxy groups (acetate, acetonide, triflate) was unsuccessful and led to complex mixtures of products.

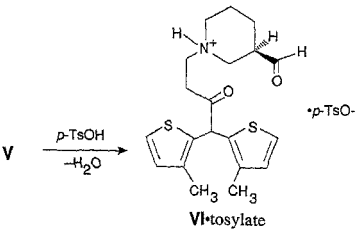
Ketotiagabine (VI)

The abundance of this product in the tablet degradates increased with the length of storage. When dihydroxytiagabine (V) was stored under acidic conditions, it was slowly converted to (VI). The degradates identified hitherto are summarized in Scheme 9. In some cases, the chromatographic degradates do not account for the total degradation (hplc assay/peak areas); other degradation routes are possible. Alternately, binding of the transient epoxide to the hydroxy or carboxy functionalities in exipients may occur. These cases are under investigation in our laboratories.

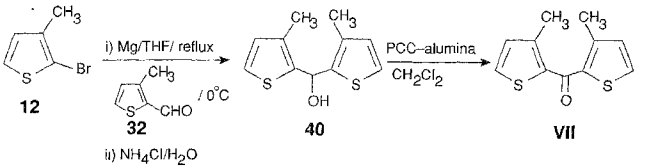


SCHEME 9.

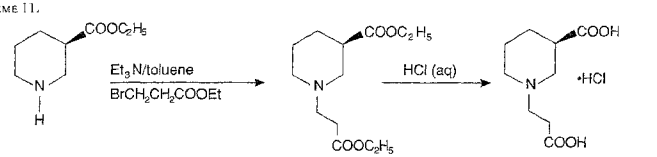
Synthesis of (VI), (VII) and (VIII) were achieved as shown in Schemes 10, 11 and 12, respectively.



SCHEME 10.



SCHEME 11.



SCHEME 12.

From this account, it will be clear that the chemistry of anti-epileptic drugs presents a fascinating area of research. Additional work on the synthesis and characterization of regioisomers of Tiagabine is in progress in our laboratory.

Acknowledgement

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