The Pictet–Spengler Condensation: A New Direction for an Old Reaction

Eric D. Cox and James M. Cook*

Department of Chemistry, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin 53201

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Contents

I. Introduction

The Pictet–Spengler reaction has long been an important reaction for the synthesis of both indole and isoquinoline alkaloids.[1] This condensation was discovered in 1911 by Amé Pictet and Theodor Spengler[2] when they condensed phenethylamine (**1**) with methylal to provide tetrahydroisoquinoline (**2**), as illustrated in Scheme 1.[1,3,4] The Pictet–Spengler reaction was originally utilized exclusively to prepare tetrahydroisoquinolines. Conceived upon biogenetic grounds, it was felt that isoquinoline alkaloids were formed in plants by the condensation of β-arylethyl amines with carbonyl compounds.[5] Soon after the initial report, the Pictet–Spengler reaction became the standard method for the formation of tetrahydroisoquinolines. This process was first utilized with indole bases in 1928 by Tatsui during the preparation of 1-methyl-1,2,3,4-tetrahydro-β-carboline (**4**).[6] The enzymatically catalyzed Pictet–Spengler condensation of tryptamine (**3**) with secologanin (**6**) to provide strictosidine[7–11] **7** is the key step in the biogenetic pathway to monoterpene indole alkaloids and is depicted in Figure 1. This condensation also occurs biogenetically with tryptophan (**5**).[12,13] The development of the enantiospecific Pictet–Spengler reaction in recent years has rendered this condensation an important synthetic method for the formation of macroline/sarpagine/ajmaline indole alkaloids.

The interest in the total synthesis of indole alkaloid natural products stems from their complex structures and diverse medicinal properties. For example, vincristine and vinblastine from *Catharanthus roseus*[14,15] have long been established as antitumor alkaloids[16–18] of clinical significance, while reserpine[19,20] as well as ajmaline[21] exhibit very important cardiovascular effects.[7,22] In regard to the present work, three bisindole alkaloids macralstonine acetate (**13**), macrocarpamine (**15**), and villalstonine (**16**), illustrated in Figure 2, have been found to exhibit significant activity *in vitro* against both *Entamoeba histolytica* and *Plasmodium falciparum*.[23] Macrocarpamine (**15**) was found to be the most active antiamoebic alkaloid with an activity one-fourth that of the standard drug emetine. Villalstonine (**16**) was found to be the most potent of the three alkaloids tested against *P. falciparum* and was about 15 times less potent than the antimalarial drug chloroquine. Macralstonine acetate (**13**) was much more active against both types of protozoa when compared with the parent macralstonine (**14**). The use of *Alstonia angustifolia* in traditional medicine has been well documented and is, presumably, due in large part to the activity of these three alkaloids.[24] In addition, macralstonine (**14**) has also been shown to lower blood pressure in dogs.[25,26] The development of an enantiospecific route to these indole alkaloids, based upon a common intermediate, would therefore increase the amount of material available for study as well as provide the possibility for derivatization and greater potency. In addition, entry into the optical antipode of these bases would also be possible. Research on the stereospecific Pictet–Spengler condensation has led to the enantiospecific synthesis of such an intermedi-

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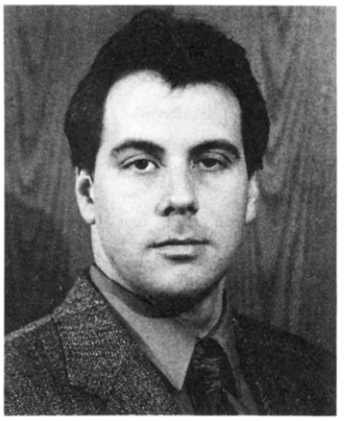
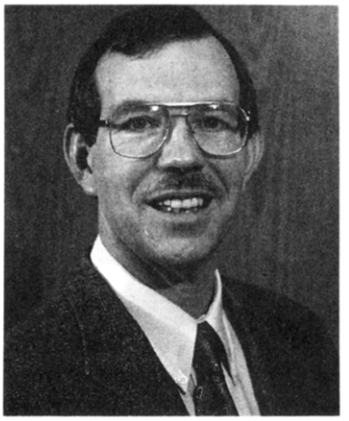


Eric D. Cox was born in Milwaukee, WI, in 1965. He received his B.S. in Chemistry from the University of Wisconsin—Madison in 1988. From 1988 until 1991 he was employed at a biomedical company in St. Paul Minnesota where he worked in technical support. He joined Professor Cook's group at the University of Wisconsin—Milwaukee in 1991 and is currently working toward his Ph.D. in organic chemistry. His research interests include the design and synthesis of anxioselective anxiolytics at the benzodiazepine receptor site. In addition he is currently working on the enantiospecific total synthesis of the bisindole alkaloid villalstonine and the indole alkaloid pleiocarpamine.



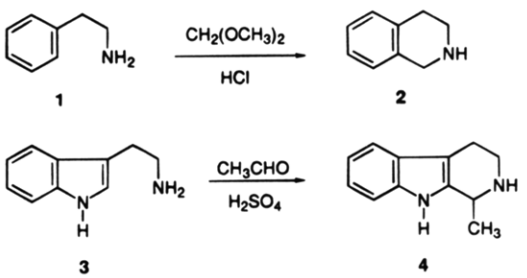
Professor James Cook received his B.S. degree in Chemistry, with honors, in 1967 from West Virginia University and his Ph.D. in 1971 from the University of Michigan. While at Michigan, he worked on the isolation and structure determination of monomeric and antihypertensive bisindole alkaloids from *Alstonia* species. During 1972–1973, he was a National Institutes of Health Postdoctoral Fellow at the University of British Columbia working on the synthesis of the antitumor alkaloids vincristine and vinblastine. He joined the faculty of the University of Wisconsin—Milwaukee in 1973 and has been Professor since 1986. An organic chemist by training, Professor Cook's interests include synthetic organic and natural products chemistry, as well as medicinal chemistry. His current research interests include the use of the chirally controlled Pictet–Spengler reaction for the total synthesis of antileukemic, antitumor, and antihypertensive indole alkaloids as well as the chemistry of reserpine, quinidine, and quinine in relation to their biological activity. This has culminated recently in an enantiospecific synthesis of the sarpagine- and ajmaline-related alkaloids, alstonerine, suaveoline, and raumacline as well as a partial synthesis of villalstonine. His group has carried out seminal studies on the use of the Weiss reaction for the synthesis of polyquinanes and is currently investigating this reaction as a source of strained polyquinenes to study bonding character in organic chemistry. The major thrust of his interest in medicinal chemistry is directed toward investigation of the structure, topology, and function of benzodiazepine (Valium) receptor subsites. β-Carbolines and diiindoles which have been prepared in this study are important tools for studying anxiety, convulsions, sleep, and memory-learning, as well as reversal of the effect of Valium–alcohol or barbiturate–alcohol overdose. Many of these analogs are Bz_1 and Bz_5 receptor subsite specific agents. Students in his group have also recently designed inhibitors of the multidrug resistance pump (MDR) of drug-resistant strains of cancer cells as well as prepared inhibitors of the indolamine 2,3-dioxygenase enzyme. This latter enzyme is involved in the pathogenesis of many inflammatory diseases including the dementia experienced by AIDS patients.

ate, the tetracyclic ketone. This intermediate will be discussed in detail later in this report. Work in this area was preceded with the discovery that the Pictet–Spengler reaction could be effected in nonacidic aprotic media as well as under the classical conditions of acid catalysis. Investigations to explore the scope of this reaction have led to an understanding of the factors which underlie the stereochemical control of this condensation. Detailed studies of the stereochemical and mechanistic factors which influence this process have been elucidated and are presented here along with the enantiospecific total synthesis of a number of indole alkaloid natural products made possible utilizing the key tetracyclic ketone mentioned above.

The Pictet–Spengler condensation has been of vital importance in the synthesis of numerous β-carbolines in addition to its use in the formation of indole alkaloids of more complex structure. Tetrahydro-β-carbolines (THBC's) have been isolated from *Virola theiodora* and other plants of South American origin and employed by Indian tribes as a botanical source of intoxicating snuffs.[27,28] In addition, some THBC's derived from tryptamine and 5-methoxytryptamine have been shown to inhibit monoamine oxidase A and bind with nanomolar affinity in the central nervous system (CNS) to serotonin receptors.[29–31] Moreover, Langer et al.[32] have reported that 6-methoxy-THBC is present in high concentrations in the human pineal gland, and that it potently inhibited the high affinity binding of [^{3}H]imipramine in human platelets. There is also a substantial amount of work which has suggested the involvement of THBC's in the etiology of alcoholism,[33,34] although, this has not yet been confirmed. The present work, however, represents the use of tryptophan alkyl esters in the Pictet–Spengler condensation and the tryptamine-related β-carbolines mentioned above will not be discussed in further detail.

The use of β-carbolines has been instrumental in the development of the inverse agonist/agonist pharmacophore of the benzodiazepine receptor (BzR) site. The BzR is located on the $GABA_A$ receptor ion channel and plays a central role in the molecular mechanisms controlling anxiety,[35,36] convulsions, memory-learning,[37] and sleep.[38] Positive or negative modulation of the $GABA_A$ receptor system by various structural classes of ligands has resulted in markedly different pharmacological effects. Agonist ligands are widely used as anxiolytics, anticonvulsants, and myorelaxants. In contrast, there are ligands which elicit pharmacological actions opposite to those of

Scheme 1



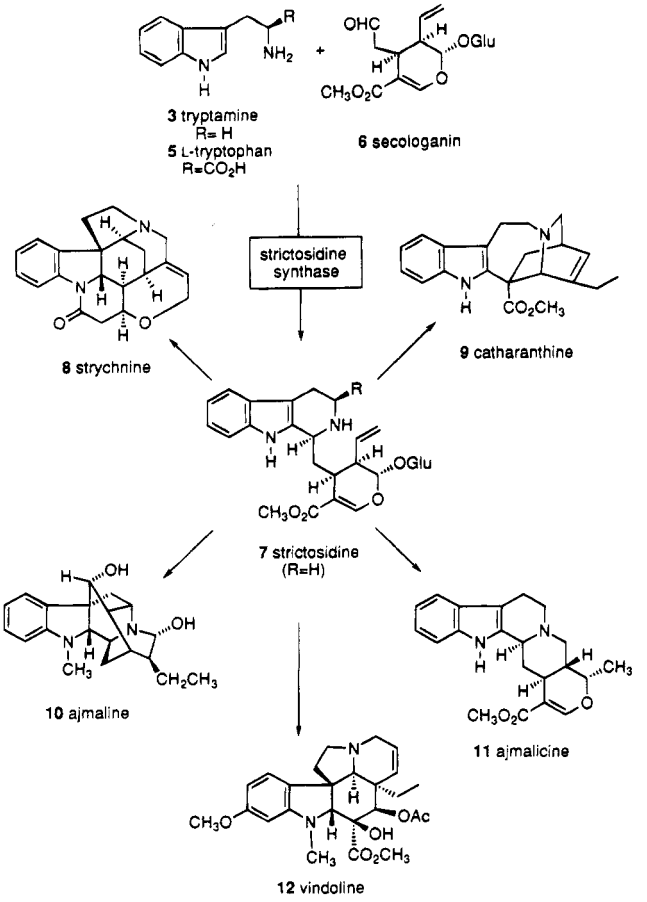


Figure 1. The strictosidine synthase-mediated Pictet–Spengler condensation of tryptamine with secologanin to provide the key biogenetic intermediate strictosidine.

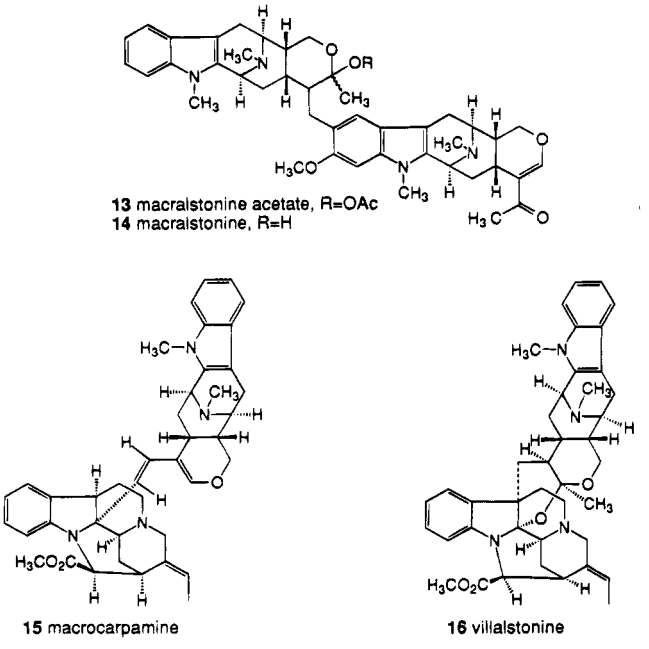


Figure 2. Several important bisindole alkaloids isolated from *Alstonia* species.

agonist ligands. These are known as inverse agonists and are represented here by β-carbolines; they exhibit anxiogenic, somnolytic, convulsant, and proconvulsant activity. At this time there is a need for selective inverse agonist and agonist ligands to be employed in the treatment of a variety of processes mediated in the CNS. More specifically, there is a need for benzodiazepine (Valium) receptor partial agonists which elicit anxiolytic and anticonvulsant effects but are devoid of the muscle relaxant and sedative side effects of classical 1,4-benzodiazepines. Moreover, partial or selective inverse agonists which enhance neuronal firing in the CNS in the absence of proconvulsant or convulsant[39–43] effects represent agents with potential therapeutic use as drugs to enhance cognition, to reverse the effects of hepatic encephalopathy, or to reverse the effects of barbiturate–alcohol-induced CNS depression (overdose).[44] A thorough understanding of the factors affecting these site(s) will permit the development of highly selective psychoactive drugs. Extensive studies using computer graphics of the inverse agonist and agonist pharmacophores coupled with synthetic results has permitted the development of the partial inverse agonist 3-EBC and the partial agonist 6-PBC as well as the Bz_1 selective antagonist βCCt. The results of these studies will be presented in this review as well as future plans regarding medicinal chemistry in the benzodiazepine receptor area.

II. Pictet–Spengler Condensations in Nonacidic Aprotic Media

In the course of work directed toward the construction of potential antihypertensive agents, the need arose for the preparation of N_b-benzyltryptophan methyl ester (**21**). This ester can be prepared by stirring tryptophan methyl ester **17** and benzaldehyde **18** in benzene at room temperature, followed by reduction of the resulting imine **19** with sodium borohydride, similar to the work of Yoneda (Scheme 2).[45] To improve the conversion of ester **17** into imine **19**, benzaldehyde and amine **17** were heated in benzene at reflux while a Dean–Stark trap was employed to remove water formed during the process. Although the imine **19** was initially observed, after prolonged heating the products of this reaction were the *cis* and *trans* diastereomers of 1-phenyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline **20** in 95% yield. This result was surprising for generally the Pictet–Spengler reaction had been carried out in a protic solvent with acid catalysts.[1,46–52] Jackson et al. earlier reported that tryptamine (**3**) and benzaldehyde (**18**) yielded only imine when heated in benzene at reflux.[53] Presumably, the Pictet–Spengler reaction with tryptophan methyl ester **17** had occurred without the aid of acid catalysts, therefore, it was decided to make a detailed study of this observation.[54]

Scheme 2

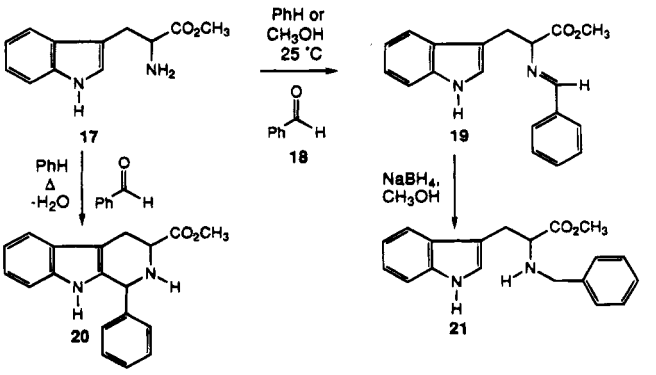
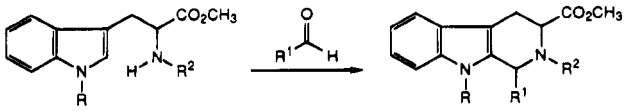
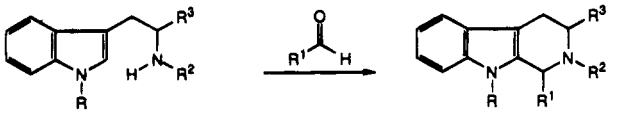


Table 1. Pictet–Spengler Cyclization of Tryptophan Methyl Ester Derivatives



amine	R	R^2	aldeyde	product	R^1	aprotic % yield	aqueous % yield
17	H	H	**18**	**20**	Ph	95	90
17	H	H	**23**	**25**	C_6H_{11}	85	73
21	H	Bn	**18**	**26**	Ph	95	
21	H	Bn	**23**	**27**	C_6H_{11}	87	
22	CH_3	Bn	**23**	**28**	C_6H_{11}	87	68
21	H	Bn	**24**	**29**	$CH_2CH_2CO_2C_2H_5$	80	50

Table 2. Reaction of Amines with Acid Labile Aldehydes



amine	R	R^2	R^3	aldehyde	R^1	product	aprotic % yield	aqueous % yield
22	CH_3	Bn	CO_2CH_3	**32**	$HC(OC_2H_5)_2$	**35**	90	<20
30	H	Bn	H	**32**	$HC(OC_2H_5)_2$	**36**	92	<25
31	CH_3	H	CO_2CH_3	**32**	$HC(OC_2H_5)_2$	**37**	65	<15
17	H	H	CO_2CH_3	**32**	$HC(OC_2H_5)_2$	**38**	62	<15
21	H	Bn	CO_2CH_3	**32**	$HC(OC_2H_5)_2$	**39**	75	<25
22	CH_3	Bn	CO_2CH_3	**33**	$C_2H_4COCH_2CH_3$	**40**	94	25
22	CH_3	Bn	CO_2CH_3	**34**	$CH_2CH_2CO_2H$[a]	**41**	97	50
22	CH_3	Bn	CO_2CH_3	**24**	$CH_2CH_2CO_2C_2H_5$	**42**	75	59

[a] Carbonyl substrate was α-ketoglutaric acid.

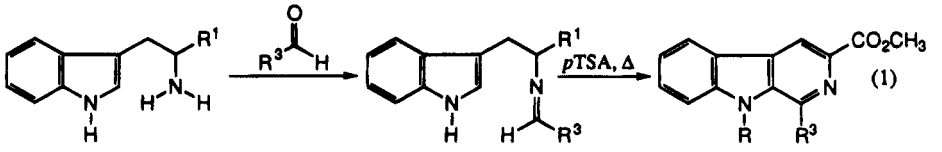
A variety of tryptophan methyl ester derivatives have been employed in this condensation and some of these are outlined in Table 1. Excellent yields of tetrahydro-β-carbolines were obtained with tryptophan methyl ester **17**, N_b-benzyltryptophan methyl ester (**21**), and N_a-methyl-N_b-benzyltryptophan methyl ester **22**. It was apparent from examination of the data in Table 1, that yields of the Pictet–Spengler reaction could be improved in nonacidic aprotic media. This was even more obvious when acid-labile aldehydes were used as substrates, as illustrated in Table 2. For example, the reaction of tryptophan methyl ester derivatives **17**, **21**, **22**, **30**, and **31** with glyoxal diethyl acetal **32** in benzene at reflux resulted in good to excellent yields of the corresponding tetrahydro-β-carbolines.[55] These same cyclizations carried out under aqueous acidic conditions resulted in considerably poorer yields of the tetrahydro-β-carbolines. The yields were generally two to three times better under nonacidic aprotic conditions than those obtained in an aqueous acidic medium. From the data in Table 2 it was obvious the Pictet–Spengler reaction could be extended to include aldehydes containing functionality such as acetals, esters, amides, and acetonides, heretofore too labile to be practical for this condensation.

Jackson and co-workers have extensively studied two possible pathways for the Pictet–Spengler reaction between tryptamine derivatives and carbonyl compounds.[53,56,57] Regardless of which path occurs, it is the electrophilic nature of the imine double bond which is the driving force of the cyclization.[54] Performing the reaction in nonacidic, aprotic media permitted a study of the correlation between the electron density on the aliphatic nitrogen atom with the ease of cyclization since protonation of the nitrogen atom by solvent was no longer a complicating factor.

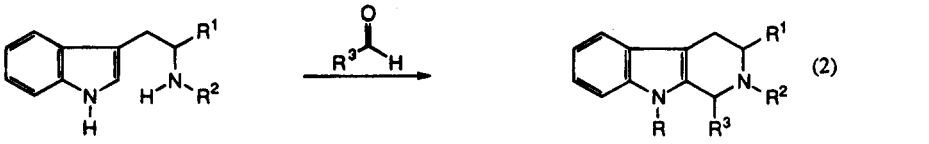
The reaction of tryptophan methyl ester (**17**) with benzaldehyde (**18**) in benzene at reflux resulted in the tetrahydro-β-carboline **20** in 90% yield. Conversely, the reaction of tryptamine (**3**) under the analogous conditions resulted only in the formation of the Schiff's base **50** in quantitative yield, as illustrated in Table 3. These results can be rationalized by examination of the pK_a values of the two bases: tryptamine, $pK_a = 10.2$,[58] tryptophan methyl ester, $pK_a = 7.29$.[59] The tryptophan methyl ester imine intermediate **19** is clearly more electrophilic than the imine **50**, formed from tryptamine (**3**). In fact, even in the presence of small amounts of tryptophan or tryptophan methyl ester hydrochloride the tryptamine intermediate **50** failed to cyclize.

It was reported in 1974 by Hamaguchi et al.[46] that the condensation of salicylaldehyde (**43**) with tryptophan methyl ester (**17**) in acidic media provided the Pictet–Spengler product **46** (Table 3, eq 2) as a mixture of the *cis* and *trans* isomers **46a,b**, albeit in 3.5% overall yield. This same condensation carried out in benzene or toluene at reflux (Table 3, eq 1) provided only the imine **45**. This imine was so unreactive in fact, that cyclization was only achieved by heating in toluene at reflux for 48 h in the presence of *p*-toluenesulfonic acid. These harsh reaction conditions failed to provide the tetrahydro-β-carboline, but rather led to the fully aromatic β-carboline **48** (Table 3, eq 1). These results can be rationalized by the mesomeric effect of the phenolic oxygen atom. The electron release by this atom rendered the imine double bond of tryptamine **45** less

Table 3. Influence of Electrophilic Character of Imine on Cyclization



17, R^1=CO_2CH_3 **18**, R^3=Ph, r.t. **19**, R^1=CO_2CH_3, R^3=Ph **48**, R^3=*o*-hydroxyphenyl
3, R^1=H **18**, R^3=Ph, Δ **45**, R^1=CO_2CH_3, R^3=*o*-hydroxyphenyl
17, R^1=CO_2CH_3 **43**, R^3=*o*-HOPh **50**, R^1=H, R^3=Ph



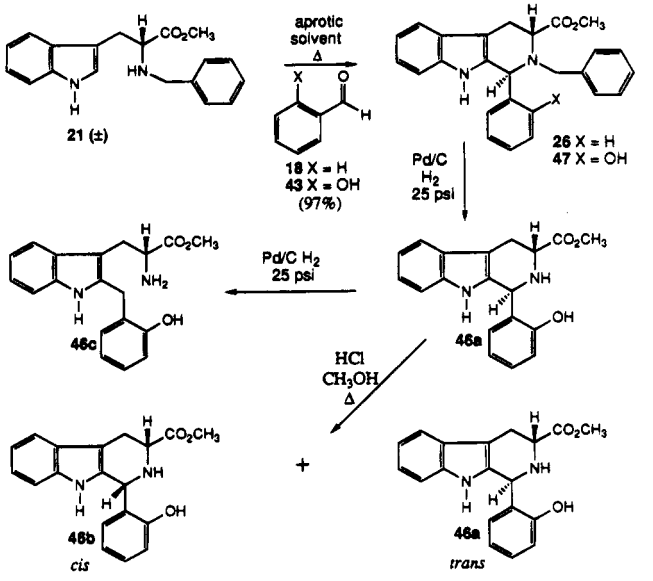
17, R^1=CO_2CH_3, R^2=H **18**, R^3=Ph, Δ **20**, R^1=CO_2CH_3, R^2=H, R^3=Ph
17, R^1=CO_2CH_3, R^2=H **43**, R^3=*o*-HOPh **46**, R^1=CO_2CH_3, R^2=H, R^3=*o*-hydroxyphenyl
21, R^1=CO_2CH_3, R^2=Bn **43**, R^3=*o*-HOPh **47**, R^1=CO_2CH_3, R^2=Bn, R^3=*o*-hydroxyphenyl
17, R^1=CO_2CH_3, R^2=H **44**, R^3=*o*-acetylphenyl **49**, R^1=CO_2CH_3, R^2=H, R^3=*o*-acetylphenyl
30, R^1=H, R^2=Bn **18**, R^3=Ph, Δ **51**, R^1=H, R^2=Bn, R^3=Ph

amine	R^1	R^2	aldehyde (Δ)	R^3	product	medium	% yield	amine	R^1	R^2	aldehyde (Δ)	R^3	product	medium	% yield
17	CO_2CH_3	H	**18**	Ph	**20**	benzene	90	**17**	CO_2CH_3	H	**43**	*o*-hydroxyphenyl	**48**	toluene/ pTSA	5
17	CO_2CH_3	H	**43**	*o*-hydroxyphenyl	**45**	toluene	100	**17**	CO_2CH_3	H	**44**	*o*-acetylphenyl	**49**	benzene	40
17	CO_2CH_3	H	**43**	*o*-hydroxyphenyl	**46**	methanol/ water	3.5	**3**	H	H	**18**	Ph	**50**	benzene	100
21	CO_2CH_3	Bn	**43**	*o*-hydroxyphenyl	**47**	benzene	62	**30**	H	Bn	**18**	Ph	**51**	benzene	98.5
21	CO_2CH_3	Bn	**43**	*o*-hydroxyphenyl	**47**	toluene	97	**17**	CO_2CH_3	H	**18** (rt)	Ph	**19**	benzene	100

electrophilic in comparison to the same bond in imine **19**. Further support for the hypothesis was obtained by the formation of *cis* and *trans* isomers of *o*-acetoxyphenyl-1,2,3,4-tetrahydro-β-carboline **49** prepared in moderate yield (Table 3) from the condensation of acetylsalicylaldehyde (**44**) and tryptophan methyl ester (**17**) in benzene at reflux. In this case the electron-withdrawing effect of the acetyl group retarded the electron-donating capability of the phenolic oxygen atom and permitted cyclization to occur in the desired manner to provide the tetrahydro-β-carboline represented by **49**.

As alluded to earlier, the electrophilicity of the imine double bond was the limiting factor which determined whether cyclization would be achieved in nonacidic aprotic medium. With this in mind, the use of the N_b-benzyl group would provide an electrophilic iminium ion intermediate which should readily undergo cyclization. In addition, the benzyl moiety could be easily removed later by catalytic hydrogenation. The results of this investigation are shown in Scheme 3 and the yields are listed in Table 3. The reaction of N_b-benzyltryptophan methyl ester (**21**) with salicylaldehyde (**43**) in toluene at reflux provided tetrahydro-β-carboline **47** in 97% yield. Catalytic debenzylation of **47** with Pd/C at 25 psi (H_2) gave the *trans*-tetrahydro-β-carboline **46a** in 55–70% yield accompanied by the 2-benzyl derivative **46c**, the product of a second hydrogenolysis. In every case in which Sandrin et al.[55] examined the substitution of a benzyl group on the aliphatic nitrogen atom of either tryptamine (**3**) or tryptophan methyl ester (**21**), the effect has been to speed the rate of the cyclization and to improve the yield. Furthermore, cyclization of an imine which contained an N_b-isopropyl group

Scheme 3



(Table 1) resulted in lower yields of product which may be due to steric effects, electronic effects, or presumably, to a mixture of both effects.[54,60]

The utilization of high boiling solvents such as cumene or diglyme to facilitate the Pictet–Spengler cyclization without aldehyde decomposition is an advantage of the reaction in aprotic nonacidic media.[46,52] The synthetic potential of the Pictet–Spengler cyclization in aprotic media with either tryptophan methyl ester derivatives or N_b-benzyltryptamines is quite general and can be employed with a variety of aldehydes which may contain acid-labile functional groups. The optimum conditions for

this modification appear to be the use of N_b-benzyl derivatives in benzene or toluene at reflux. Care must be taken to assure that the boiling point of the aldehyde is higher than that of the solvent; aldehydes such as acetaldehyde readily distill off into the Dean–Stark trap and give poor yields of tetrahydro-β-carbolines. In these cases the use of a sealed tube provides improved yields.[61,62] For substances that are not soluble in benzene or toluene, dioxane can be added to the reaction medium.

A. Stereochemical Assignment of 1,3-Disubstituted 1,2,3,4-Tetrahydro-β-carbolines

The use of nonacidic, aprotic media in the Pictet–Spengler reaction was a significant improvement upon standard reaction conditions for this condensation. Because this process resulted in the formation of *cis* and *trans* isomers it became necessary to establish a general method for the assignment of stereochemistry to differentiate between these diastereomeric 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines.

The use of ORD/CD had been successfully employed by Brossi et al.[63] in the 1-methyl-3-carboxyl series; however, accurate assignments using this technique required pure samples and such diastereomers were often difficult to separate. Inconsistent results often occurred with proton NMR due to the overlap of signals from the protons located at C-1. In addition, the assignment of stereochemistry by ^{1}H NMR for the *cis* and *trans* isomers in the 1-phenyl-3-methoxycarbonyl series (Scheme 4) has led to conflicting assignments.[46,64] Chemical correlation (preferential cyclization of only the *cis* isomer) of stereochemistry carried out by Smith[13] required the synthesis of specific compounds with the proper functionality at carbon atoms 1 and 3 to permit cyclization. This chemical approach was considered too laborious to be employed in a general sense, consequently attention turned to ^{13}C NMR spectroscopy.

Assignments in the 1-phenyl-3-methoxycarbonyl series using carbon-13 magnetic resonance spectroscopy had been reported in preliminary fashion by Sandrin et al. in 1976.[65] Moreover the use of ^{13}C NMR for the stereochemical assignment and structure proof of a number of yohimbinoid and ajmalicinoid alkaloid systems was also published in that same year by Wenkert et al.[66] It became clear that carbon NMR spectroscopy would be devoid of the complications observed in proton NMR; therefore, studies were initiated to determine the limits of the method described earlier.[67] The ^{13}C NMR method developed by Sandrin et al.[65] employed the well-documented compression effect[68] observed in ^{13}C NMR spectroscopy, and will be illustrated only briefly for the *cis*- and *trans*-tetrahydro-β-carbolines **20a** and **20b.**

The *cis* and *trans* isomers were prepared using a Pictet–Spengler condensation between tryptophan methyl ester (**17**) and benzaldehyde (**18**) in nonacidic aprotic media[54] or in an aqueous acidic media (Scheme 4). The *cis* and *trans* diastereomers were isolated, separated, and the ^{13}C NMR spectrum of each was recorded. The signal assignments were made on the basis of nuclear Overhauser effects (nOe), correlations with known compounds, and off-resonance decoupled spectra. The carbon signals for carbon atoms C-1 and C-3 for the compounds in Scheme 4 are illustrated in Table 4. The carbon signals assigned to the *cis* isomer **20a** [C-1 (58.7 ppm) and C-3 (56.9 ppm)] were downfield relative to those of isomer **20b** [C-1 (54.9 ppm) and C-3 (52.3 ppm)] which was then assigned as the *trans* diastereomer. Conformational analysis and examination of molecular models indicated that of the two possible twist chair conformations (A or B, Scheme 5) for the *trans* isomer **20b**, conformer A should represent the structure of the more stable species due to the 1,4-gauche interaction between the hydrogen atom at C-1 and the substituent located at C-3 in conformer B. More importantly,

Scheme 4

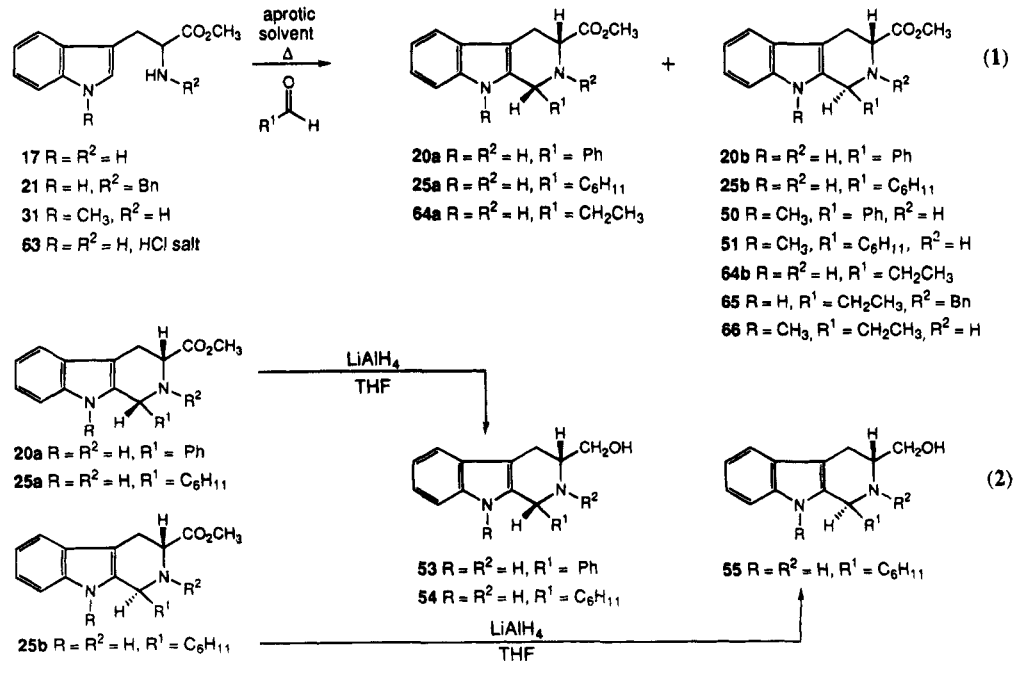
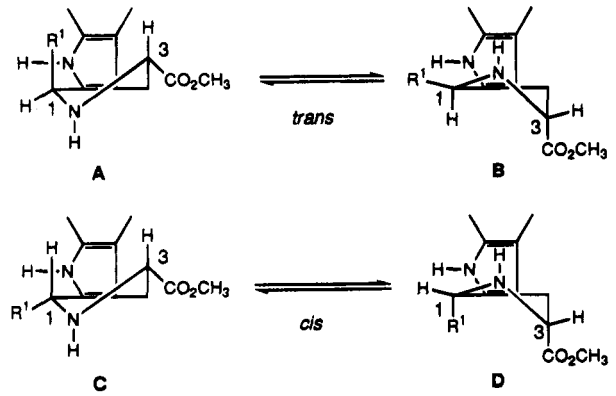


Table 4. ^{13}C NMR Signals for C-1 and C-3

compound	stereochemical configuration	^{13}C signal C-1 (ppm)	^{13}C signal C-3 (ppm)
20a	*cis*	58.7	56.9
20b	*trans*	54.9	52.3
25a	*cis*	57.7	56.6
25b	*trans*	55.4	53.4
50	*trans*	54.8	51.1
51	*trans*	54.5	52.8
52	*cis* C(1) = ^2H		57.0
53	*cis*	58.8	56.4
54	*cis*	57.9	55.8
55	*trans*	55.9	51.3
56	*cis*	53.1	56.5
58	*trans*	52.8	52.7
60	*trans*	49.9	52.9
64a	*cis*	53.8	56.5
64b	*trans*	51.7	52.8
66	*trans*	51.1	52.2

Scheme 5

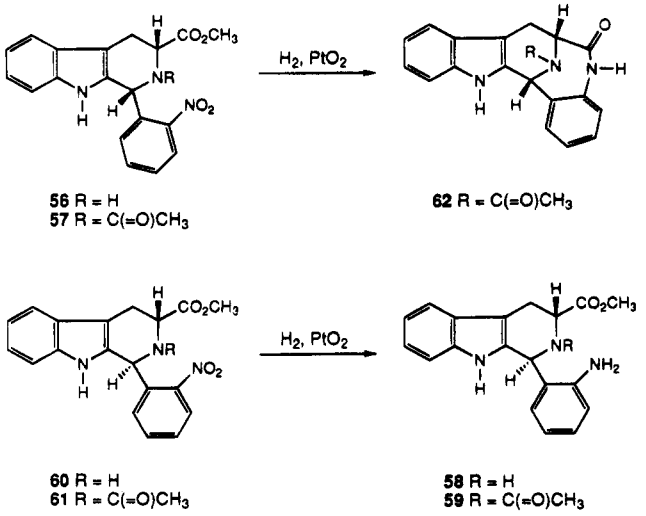


conformer B undergoes an unfavorable interaction between the indole N_a-H and the equatorial phenyl group located at C-1 [$A^{(1,2)}$ strain].[69] The methoxycarbonyl group at C-3 is located in the equatorial position in the more stable conformer A while the substituent at C-1, although now in the axial position, is devoid of the interaction between the substituent at C-1 and the N_a-indole hydrogen atom. A similar analysis of the *cis* diastereomer **20a** led to the conclusion that conformer C (with no ^{13}C-γ gauche effect between atoms bonded to carbons 1 and 3) should be much more stable than conformer D. Consequently, it would be expected that the signals for carbon atoms 1 and 3 in *trans* isomer **20b** (with a ^{13}C-γ effect) would appear at a higher field in the carbon NMR spectrum, respectively, than those of the corresponding *cis* isomer. This was found to be the case in 1-cyclohexyl-3-methoxycarbonyl bases **25a** (*cis*) and **25b** (*trans*) (Scheme 4) as well as the 1-phenyl compounds discussed above. Additional data were required, moreover, to support the assumption that the assignment of *cis* and *trans* configurations to the above indoles was correct and that the 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines were not subject to other subtle influences which might lead to erroneous conclusions.

In this regard N_a-methyltryptophan methyl ester **31** was heated at reflux in an aprotic solvent with either benzaldehyde (**18**) or cyclohexanecarboxyaldehyde (**23**) which provided the corresponding 1-substituted derivatives **50** and **51**, respectively, in excellent yields. Examination of molecular models had indicated that the $A^{(1,2)}$ strain between the substituent at C-1 and the N_a-methyl group would permit only formation of the *trans* diastereomer under these conditions. In agreement with this, only one diastereomer was isolated from each synthesis (>85% yield); furthermore, the carbon signals for *trans* diastereomer **50** [C-1 (54.8 ppm) and C-3 (51.1 ppm)] were virtually identical to those of the *trans* isomer **20b** [C-1 (54.9 ppm) and C-3 (52.3 ppm)]. This same phenomenon was observed in the case of *trans* diastereomer **51**. While the latter two experiments demonstrated indirectly the validity of this approach, additional experiments were necessary to accurately determine which signals in Table 4 were due to the resonance at C-1.

For this purpose tryptophan methyl ester **17** was condensed with deuterobenzaldehyde[70] in the presence of p-toluenesulfonic acid (*p*TSA) to provide a mixture of two components, the R_f values of which were identical to those of phenyl analogs **20a** and **20b**. These diastereomers were separated by chromatography and the ^{13}C NMR spectrum (except at C-1) and melting point of the more accessible *cis* compound **52** (Table 4) were found to be in complete agreement with a structure such as **20a** (**52** 1_d). The carbon spectrum of **52** 1_d was devoid[71,72] of the signal at 58.7 ppm due to C-1 in **20a**; therefore, it was clear that the signal at 58.7 ppm in **20a** resulted from the carbon atom at position 1 while that at 56.9 ppm corresponded to carbon atom C-3. To further corroborate this assignment and to extend this to the cyclohexyl bases **25a** and **25b**, *cis*-1-phenyl-**20a**, *cis*-1-cyclohexyl-**25a**, and *trans* 1-cyclohexyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (**25b**) were treated with $LiAlH_4$ in THF. The 3-hydroxymethyl-substituted bases were isolated and subjected to ^{13}C NMR spectroscopy (Table 4). In all cases [**20a** → **53** (δ −0.5 ppm), **25a** → **54** (δ −0.8 ppm), **25b** → **55** (δ −2.1 ppm) the signal for the carbon atom at C-3 was shifted upfield by 0.5 ppm or more; the same phenomenon had been observed on going from methyl acetate to ethanol (δ −1.7 ppm).[73] Having now determined the chemical shifts for carbon atoms 1 and 3 in the 1-phenyl and 1-cyclohexyl series, direct chemical proof was required to establish that the ^{13}C NMR spectroscopic method was correct in the most unequivocal sense. Smith and co-workers[13] had shown that *cis*-1-(hydroxymethyl)-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (as the N_b-amide) would cyclize to a lactone, while the *trans* isomer would not. Spenser[74] had demonstrated, however, that these 1-hydroxymethyl derivatives were quite labile and consequently the *cis*- and *trans*-1-(*o*-nitrophenyl) bases depicted in Scheme 6 were chosen for study. A mixture of *cis* and *trans* diastereomers were obtained from the Pictet–Spengler condensation of **17** with *o*-nitrobenzaldehyde, and the two compounds (**56** and **60**) were carefully separated by chromatography. The assignment of stereochemistry was initially based upon the carbon spectrum of these two molecules. When the *trans* isomer was subjected to catalytic hydrogenation, only 2-amino derivative **58** was isolated; however, the *cis* diastereomer **56**, under analogous conditions gave a complex mixture of products. According to Hobson[75] the formation of

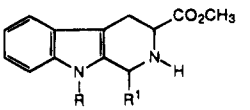
Scheme 6



an amide would flatten the twist chair conformation of ring C of a tetrahydro-β-carboline and would bring the two groups *cis* 1,3-disposed into closer proximity to permit more facile cyclization. In view of this, both *o*-nitro bases **56** (*cis*) and **60** (*trans*) were converted into the corresponding acetamide derivatives **57** and **61**, respectively, by treatment with acetic anhydride and pyridine. The *trans* amide **61** gave only amide **59** when treated with hydrogen and PtO_2 while the *cis* isomer under the same reaction conditions was converted into the pentacyclic lactam **62** in 30% yield. This latter transformation clearly represented chemical proof of the validity of the ^{13}C NMR method for stereochemical assignments when position 1 was substituted with an aryl group.

Attention now turned to tetrahydro β-carbolines which contained smaller substitutents at C-1. In this vein, it was decided to synthesize the *cis* and *trans* diastereomers of 1-ethyl-substituted tetrahydro-β-carboline (**64a,b**) from amino ester **63** and propionaldehyde **68**, as illustrated in Scheme 4. Brossi[63] had shown that 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines which have a methyl group at C-1 are found predominantly as the *cis* isomer. It should be noted, however, that extrapolation to larger groups can lead to erroneous conclusions.[64] The condensation of **63** with propionaldehyde (**68**) in acidic solution provided the 1, 3-disubstituted 1,2,3,4-tetrahydro-β-carbolines as a mixture of *cis* and *trans* isomers represented by **64a** and **64b**, respectively. The diastereomeric ratio depicted in Table 5 suggested that $A^{(1,2)}$ strain was significant enough to favor the *trans*-1-ethyl diastereomer **64b**. Separation of the two diastereomers followed by examination of their ^{13}C NMR spectra led to the initial assignment of stereochemistry. Following the initial assignment of stereochemistry attempts were made to corroborate the findings by another method. Chemical correlation of the *cis* isomer (via cyclization) was not possible, however, Ungemach et al.[76] had reported earlier that N_b-benzyltryptophan methyl ester (**21**) reacted with either salicylaldehyde (**43**), glyoxal diethyl acetal (**32**), or propionaldehyde (**68**) to provide only the *trans*-1,3-disubstituted β-carboline (e.g. **65**, illustrated in Scheme 4). When the *trans* base **65** was subjected to catalytic hydrogenation this resulted

Table 5. Ratio of *Cis/Trans* Diastereomers



compound	R	R^1	diastereomeric ratio[a] (*cis*:*trans*)	ref
20a, 20b	H	Ph	40:60	46
25a, 25b	H	C_6H_{11}	40:60	46
50	CH_3	Ph	0:100	46
51	CH_3	C_6H_{11}	0:100	46
56, 60	H	2-$NO_2C_6H_5$	25:75	71
64a, 64b	H	CH_2CH_3	43:57	56
66	CH_3	CH_2CH_3	0:100	56

[a] In a pair of diastereomers the second number represents the *trans* isomer in this table.

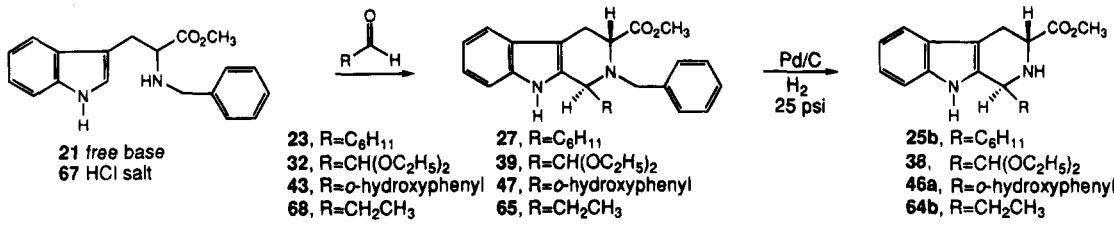
in the formation of 1,3-disubstituted β-carboline **64b** as the only isolable material. In addition, excellent agreement was observed when comparison of the chemical shifts for carbon atoms 1 and 3 in the spectrum of **64b** (51.7, 52.8 ppm) was made with the signals in the ^{13}C NMR spectrum of *trans* **66** [51.1, 52.2 ppm (N_a-methyl)]. More importantly, the *trans* stereochemistry of the 1,3-disubstituted 1,2,3,4-tetrahydro-β-carboline **64b** was confirmed by X-ray crystallography.[77]

In all of the examples examined here, the signals for C-1 and C-3 of the *cis* diastereomers are clearly distinct from the analogous resonance lines for the *trans* diastereomers. The enantiospecific synthesis of indole alkaloids in the macroline/sarpagine/ajmaline series rests on the accurate assignment of the stereogenic centers at C-1 and C-3 in the corresponding 1,3-disubstituted tetrahydro-β-carbolines. Although Bailey et al. have reported a ^{13}C NMR method to differentiate between *cis* and *trans* diastereomers in the N_a-hydrogen-N_b-benzyl series, this method is not 100% effective as noted by the authors.[78] Moreover, Tóth et al. have examined this method for stereochemical assignments and also found exceptions.[79] To date, accurate stereochemical assignments for the *cis*- and *trans*-1,3-disubstituted N_b-benzyltetrahydro-β-carbolines in the above series can only be made in 100% of these cases by removal of the N_b-benzyl group [catalytic debenzylation (CTH): Pd/C, NH_4CO_2H, CH_3CH_2OH] followed by identification of the diastereomers by the ^{13}C NMR method developed by Sandrin and Ungemach.[65,77] In addition, this ^{13}C NMR technique[77] provided for the first time a facile method to study the effects of $A^{(1,2)}$ strain in the determination of configurational preference of 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines in the Pictet–Spengler cyclization. With these results in hand, attention now turned to the stereospecific synthesis of *trans*-1,3 disubstituted-1,2,3,4-tetrahydro-β-carbolines. Many of these tetrahydro-β-carbolines have demonstrated interesting biological activity or are important intermediates in the total synthesis of indole alkaloids.

B. Stereospecific Synthesis of *trans*-1,3-Disubstituted 1,2,3,4-Tetrahydro-β-carbolines

Several groups[13,46,52,54,64] had earlier investigated the ratio of *cis*/*trans* isomers in the Pictet–Spengler

Scheme 7



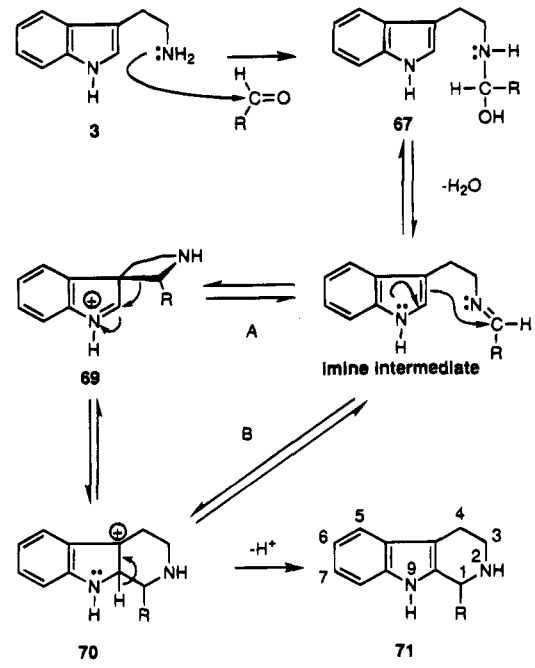
reaction. In all of the reactions examined previously, mixtures of *cis* and *trans* isomers had been reported. During the preparation of 1,3-disubstituted β-carbolines for ^{13}C NMR studies[77] Ungemach had discovered that the reaction of N_b-benzyltryptophan methyl ester (**21**) with salicylaldehyde (**43**) in toluene at reflux provided a single diastereomer, the Pictet–Spengler product **47** in 97% yield (Table 3). Examination of the ^{13}C NMR spectrum confirmed that only one diastereomer had been formed in this process; however, steric interactions in this 1,2,3-trisubstituted β-carboline were too complex to permit an unequivocal assignment using ^{13}C NMR spectroscopy.[77]

Removal of the N_b-benzyl function of this indole, however, permitted comparison of the properties of this base with those of authentic *cis*- or *trans*-1,3-disubstituted β-carbolines previously prepared by an independent route.[54] In this vein, the 2-benzyl derivative was subjected to catalytic hydrogenation (10% Pd/C, 25 psi) which resulted in a 75% yield of *trans*-tetrahydro-β-carboline **46a** (Scheme 7). Earlier it had been reported[46,54] that treatment of tryptophan methyl ester with salicylaldehyde under the same conditions resulted in a mixture of *cis* and *trans* isomers (**46b,a**). The N_b-benzyl group clearly directed this condensation in a *trans* stereospecific manner.[60,76] This represented the first completely stereoselective result in the N_a-H tetrahydro-β-carboline methyl ester series. The possibility that hydrogen bonding (hydroxyl group of the salicyl moiety **43**) might play a role in the stereoselectivity of this reaction led to the use of a second aldehyde, devoid of the hydroxyl group.

Cyclohexanecarboxaldehyde (**23**) was heated with N_b-benzyltryptophan methyl ester (**21**) in benzene at reflux to provide the tetrahydro-β-carboline (**27**) in 87% yield (Scheme 7). Again, examination of the ^{13}C NMR spectrum of this base indicated the presence of only one isomer in the reaction mixture, therefore, it was clear that the hydroxyl group played no role in directing the stereochemical outcome of the condensation. Catalytic debenzylation of **27** gave a 70% yield of *trans*-1-cyclohexyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (**25b**). As in the previous example this reaction carried out in the absence of the N_b-benzyl function resulted in the formation of *cis* and *trans* isomers.[54] The N_b-benzyl sequence had again occurred with 100% *trans* diastereoselectivity.

Previous studies had shown that $A^{(1,2)}$ strain between position 1 of the tetrahydro-β-carboline and the N_a-substituent was the dominant factor in determining the *cis/trans* ratio in the Pictet–Spengler reaction in the N_b-H series.[67,77] With this in mind it was decided to condense smaller aldehydes with amine **21** and examine the *cis/trans* ratio. Glyoxal diethyl

Scheme 8



acetal (**32**) was condensed with methyl ester **21** in refluxing benzene to furnish the desired tetrahydro-β-carboline **39** in 75% yield (Scheme 7). Catalytic debenzylation of the base provided the 1,3-disubstitued β-carboline **38** in excellent yield. Careful examination of the ^{13}C NMR spectral data showed no evidence for the *cis* isomer. Even more important was the result when propionaldehyde (**68**) was treated with the hydrochloride salt of N_b-benzyltryptophan methyl ester (**67**) in methanol/water at reflux (cold finger condenser) for 48 h. Three compounds were isolated from this reaction, one of which was the desired *trans*-N_b-benzyl derivative **65** (46%) and the other two products appeared to arise from attack of the indole nitrogen atom on the aldehyde. Treatment of the base **65** with hydrogen over palladium on carbon yielded the *trans*-1-ethyl-3-(methoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (**64b**) in 97% yield. These results clearly indicated the stereospecificity of this sequence was due largely to the effect of the N_b -benzyl substituent and not solely the result of $A^{(1,2)}$ strain on the system.[69] This result was later confirmed when ester **21** was condensed with butyraldehyde in a sealed tube to provide the *trans* diastereomer with 77:23 (*trans/cis*) diastereoselectivity.[61,62]

An examination of the mechanism of this condensation with regard to diastereoselectivity is not a simple matter. The Pictet–Spengler reaction (see **67–71**) has generally been thought to proceed via a spiroindolenine intermediate[5,56,78] as shown in Scheme 8 (path A), although Casnati[80] has shown that

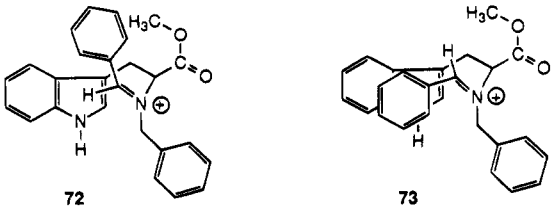


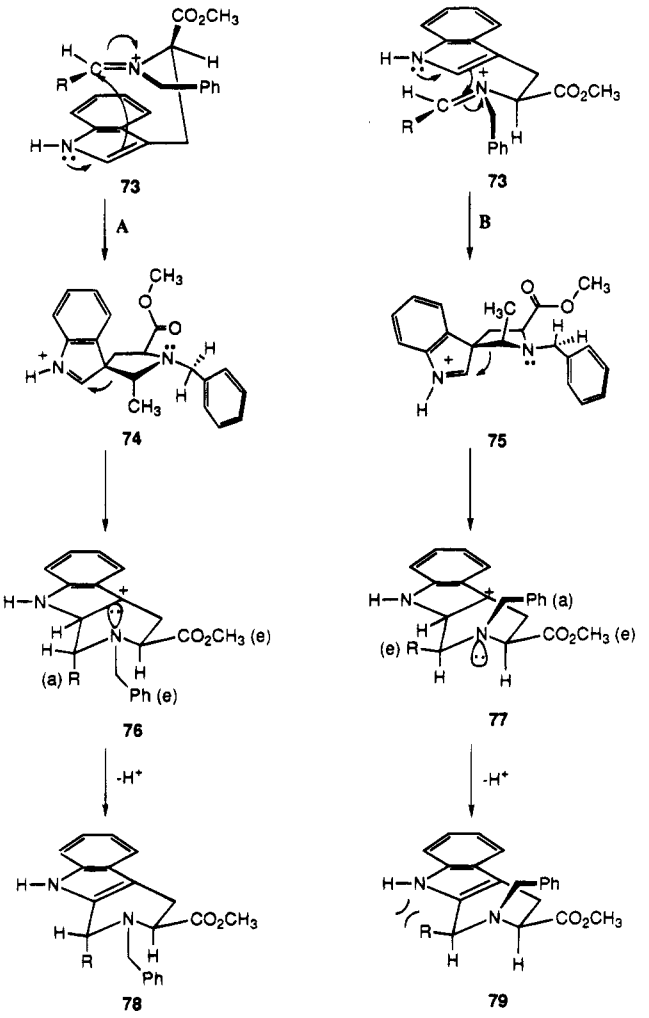
Figure 3. Steric interactions in the two possible iminium ion intermediates.

cyclization can occur by direct attack at position 2 of the indole (path B) when very reactive electrophiles are employed. The benzyl imine intermediate (when using N_b-benzyltryptophan) can certainly be considered a reactive electrophile; therefore, both pathways must be considered when discussing the stereochemical outcome of this cyclization. Molecular models have been used to examine the steric interactions on the benzyl iminium ion intermediates **72** and **73** (Figure 3). These steric interactions which would develop in the transition state between the indole moiety and the phenyl group (or R group) in stereoisomer **72** are much higher in energy than the interactions between the indole group and the hydrogen atom of intermediate **73**. In addition, attack of the indole double bond of **72** (*Z* isomer) would result in a 1,3-interaction between the group at position −1 and the methyl ester in the transition state as the electronic character of the iminium ion approaches sp^3 hybridization. Conversely, this same unfavorable interaction would not develop in the *E* isomer **73**; therefore, stereoisomer **73** is believed to be the favored intermediate in this cyclization.

The second factor which introduces stereospecificity into this sequence is the stereoelectronic control in attack of the indole double bond on the benzylium ion **73**. Attack can occur from either C-2, or C-3 (see Scheme 9), but is expected to occur antiperiplanar to the methyl ester, similar to the attack of hydroxide ion on imidate salts reported by Deslongchamps.[81]

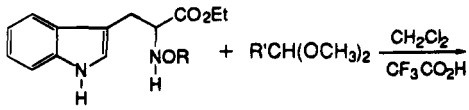
The structures which result from attack of the indole double bond at C-3 on the benzylium ion **73** from the bottom face (A) and the top face (B) of the carbon−nitrogen double bond are illustrated in Scheme 9. Intermediate **75** is clearly more crowded due to the *syn* alignment of the substituents at C-1, -2, and -3 than the *anti* spiroindolenine intermediate depicted in conformer **74**. Attack of the indole 2,3-double bond on imine **73** is believed to occur from the face opposite the ester function which minimizes interaction with this group during cyclization. Examination of Scheme 9 shows the stereospecificity of this sequence is even more obvious when intermediates **76** and **77** are examined. Spiroindolenine intermediate **74** results from the intramolecular attack (in a stereoelectronic sense) on imine **73** from the bottom face of the iminium ion, anti to the ester function. The result of this attack is occupation of the pseudoequatorial position with the benzyl group while the phenyl group at C-1 would be pseudoaxial. Rearrangement of this intermediate leads to carbocation **76** which contains the two equatorial groups accompanied by the 1-axial substituent. Comparatively, attack on imine **73** from the top face of the iminium ion double bond would result in the more crowded spiroindolenine intermediate **75** which contains (after rearrangement) an axial benzyl function, an equatorial methoxycarbonyl group, and the equatorial 1-phenyl moiety. The *cis* diastereomer **77** is clearly the less stable diastereomer since the 2-benzyl function occupies an axial position in the transition state. In addition, the *cis* diastereomer **77** suffers from the additional unfavorable interaction between the equatorial substituent at position 1 and the indole N_a-H function [$A^{(1,2)}$ strain]. It is therefore the combined influence of stereoelectronic control and conformational interactions which leads to complete stereospecificity in this Pictet−Spengler reaction to provide the *trans* isomer **78**.

Scheme 9



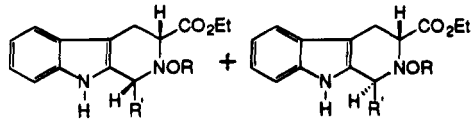
Evidence suggested[60] that the N_b-benzyl group when used in conjunction with large aldehydes resulted in the stereospecific formation of *trans*-1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines. It was discovered, however, during the work of Ottenheijm[82] on the synthesis of eudistomin alkaloids,[83,84] that the reaction of N_b-(benzyloxy)tryptophan ethyl ester (**81**) with acetals **82** and **83** (Table 6) did not proceed with the same stereospecificity that had been earlier observed with N_b-benzyltryptophan methyl ester (**21**).[60] Investigations in our laboratory have explored the reasons for variations in the stereospecificity of the Pictet−Spengler condensation. It was therefore of interest to determine if the oxygen substituent in the N_b-hydroxyl- and N_b-(benzyloxy)tryptophan ethyl esters (**80** and **81**, respectively) was responsible for

Table 6. Condensation of N_b-Oxytryptophan Ethyl Esters with Acetals



80, R=H
81, R=CH_2Ph

82, R'=CH_3
83, R'=C_6H_5



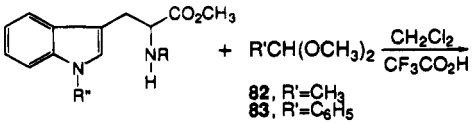
cis *trans*

84, R=H, R'=CH_3
86, R=H, R'=Ph
88, R=CH_2Ph, R'=CH_3

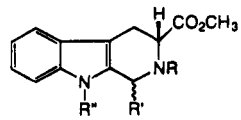
85, R=H, R'=CH_3
87, R=H, R'=Ph
89, R=CH_2Ph, R'=CH_3

			products			
reactants	R	R'	*cis*	*trans*	time (h)	yield (%)
80 + **82**	H	CH_3	**84** (67)	**85** (33)	72	95
80 + **83**	H	C_6H_5	**86** (40)	**87** (60)	6	77
81 + **82**	CH_2Ph	CH_3	**88** (50)	**89** (50)	3	96

Table 7. Condensation of N_b-Alkyltryptophan Methyl Esters with Acetals



82, R'=CH_3
83, R'=C_6H_5



				product ratios[a]		
ester	R''	R	R'	*cis* (%)	*trans* (%)	time (h)
17	H	H	CH_3	**93** (75)	**94** (25)	48
90	H	CH_3	CH_3	**95** (66)	**96** (34)	24
21	H	CH_2Ph	CH_3	**97** (16)	**98** (84)	72
91	H	CH_2CH_2Ph	CH_3	**99** (16)	**100** (84)	168
21	H	CH_2Ph	C_6H_5	**101** (0)	**102** (100)	48
92	CH_3	CH_3	CH_3	**103** (16)	**104** (84)	288

[a] The stereochemistry and ratios of the *cis* and *trans* diastereomers were determined by ^1H and ^{13}C NMR spectroscopy.

the decreased stereospecificity when compared to the N_b-methyl- and N_b-phenethyltryptophan methyl esters (**90** and **91**, respectively), for a comparison see Tables 6 and 7.

The reactions which had been carried out in refluxing benzene were carried out under the conditions of Ottenheijm[82] in dichloromethane/trifluoroacetic acid (CH_2Cl_2/TFA). The N_b-methyl- and N_b-phenethyltryptophan methyl esters **90** and **91** were prepared to be employed as the carbon analogs of the corresponding N_b-hydroxyl- and N_b-(benzyloxy)tryptophan derivatives **80** and **81**. These derivatives permitted a comparison between the effect of size vs electronegativity (O vs C) on the stereoselectivity of the Pictet–Spengler condensation.

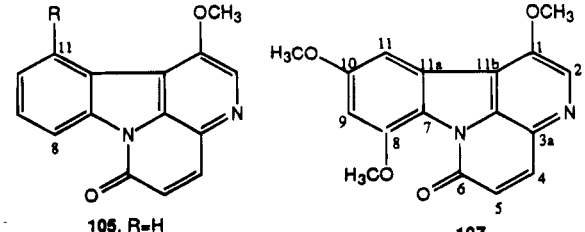
The reaction of N_b-hydroxytryptophan **80** with acetaldehyde dimethyl acetal (**82**, Table 6) resulted in substantial amounts of the *cis* diastereomer, (67:33, *cis*:*trans*) as reported.[82] This same diastereoselectivity was realized when N_b-methyltryptophan methyl ester (**90**) was condensed with acetal **82**. As expected, the size of the substituent on the N_b-nitrogen atom led to higher *trans* diastereoselectivity in the reaction as illustrated in Tables 6 and 7. The reaction of N_b-benzyloxytryptophan methyl ester (**81**) with **82** gave a 50:50 ratio of *cis*:*trans* diastereomers, while the corresponding reaction of the N_b-phenethyl base **91** led to a high degree of *trans* selectivity (16:84). Comparison of the data in Tables 6 and 7 clearly shows that the electronic contributions from the oxygen atom decrease the stereoselectivity for the isosteric tryptophan methyl esters.[85]

The studies described in the preceding section have helped to shed some light on the factors which affect the diastereoselectivity of this widely used reaction. Moreover, a short two-step sequence has been developed to prepare *trans*-1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines in 100% stereoselective fashion. Easy removal of the 2-benzyl function employed to direct the *trans* diastereoselectivity via catalytic debenzylation (CTH) renders this method useful for the diastereoselective synthesis of many different 1,3-disubstituted tetrahydro-β-carbolines and indole alkaloids of complex structure.

C. Carboxyl-Mediated Pictet–Spengler Condensation

In the last several years an increasing number of β-carboline alkaloids that contain an oxygen substituent at position 4 have been isolated.[86-90] The 4-methoxy-β-carbolines[86-88] and canthin-6-ones,[86-89] as well as several bisindoles[91] serve as representative examples. The alkaloids 1-methoxycanthin-6-one (**105**), and 1,11-dimethoxycanthin-6-one (**106**) and their congeners shown in Figure 4 have been reported to exhibit cytotoxic antileukemic activity via their inhibitory effects on DNA synthesis in GPK epithelial cells.[92,93] Oxygenation of the canthin-6-one skeleton either at position 1 (C-4 in the β-carboline numbering system) and/or ring A greatly enhanced the cytotoxic antileukemic activity of these bases.[92,93]

The synthesis of the parent 1-methoxycanthin-6-one (**105**) has been carried out and is described below. Efforts are currently focused on the synthesis of "unnatural products" such as 1,8,10-trimethoxycanthin-6-one (**107**) to investigate the mode of action of the cytotoxic activity of these unique oxygenated alkaloids. The approach to **106** or **107** requires a simple route to oxy-substituted tryptamines, the most straightforward of which was reported earlier by Abramovitch and Shapiro.[94,95] This process suffered, however, because the decarboxylation of the tryptamine-2-carboxylic acid to provide the substituted tryptamine often occurred in low yield, depending on

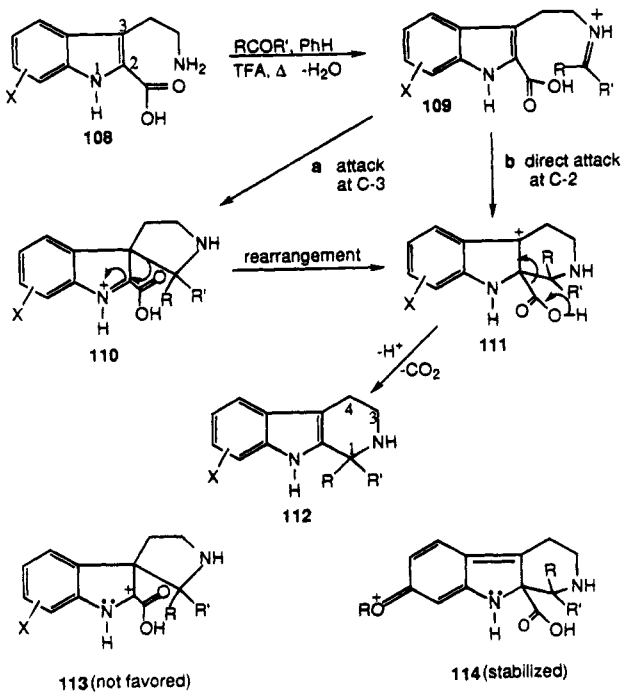


105, R=H
106, R=OCH_3

107

Figure 4. Methoxy-substituted canthin-6-ones.

Scheme 10



the nature of the substituents on the indole ring.[94,95] It was this synthetic problem which led to the development of the carboxyl-mediated Pictet–Spengler reaction.[96,97] With this in mind, the mechanism of the Pictet–Spengler reaction[5,56,80] was reviewed. As outlined in Scheme 10, if the tryptamine-2-carboxylic acid (**108**) could be encouraged to form the Schiff's base **109**, and was then heated, this might provide the spiroindolenine intermediate **110** (C-3) or the carbocation **111** [path b (C-2) or from **110**]. Loss of both the proton and the elements of CO_2 from **111** to satisfy the positive charge would provide the desired 1,2,3,4-tetrahydro-β-carboline **112**.

When a substituted tryptamine-2-carboxylic acid (**108**) was simply heated with the carbonyl compound in a solution of benzene/dioxane/trifluoroacetic acid at reflux with water removal (Dean–Stark trap) the desired tetrahydro-β-carboline was produced. The results of the condensation between various tryptamine-2-carboxylic acids and a carbonyl component are summarized in Table 8. The process appears to be quite general for simple aldehydes such as benzaldehyde **18** as well as more reactive electrophiles including α-keto acids and α-keto esters undergo the cyclization with ease. The carboxyl-mediated Pictet–Spengler cyclization employed herein represented a considerable improvement over the reported syntheses of indoles **125**–**133**, and can be extended to include many ring A-oxygen substituted tryptamine-2-acids which fail to undergo decarboxylation during attempts to convert the tryptamine-2-carboxylic acids to tryptamines.[94,95,97] This is also important for acids **119** and **120**, the decarboxylation of which is very difficult under normal reaction conditions.[97,98]

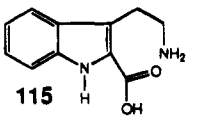
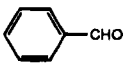
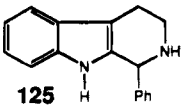
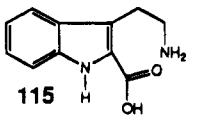
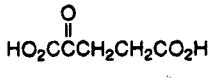
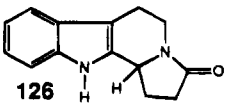
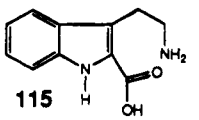
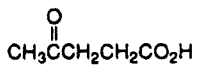
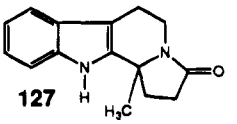
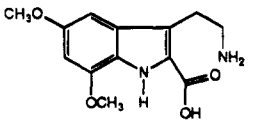
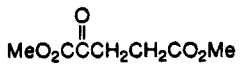
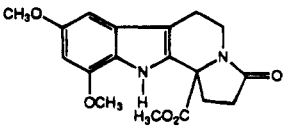
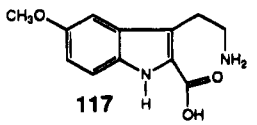
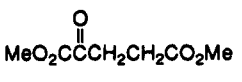
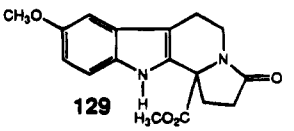
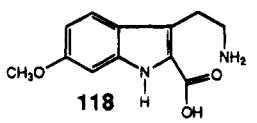
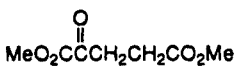
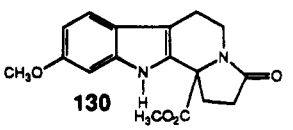
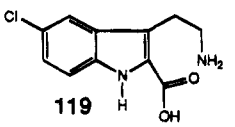
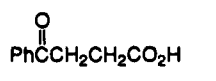
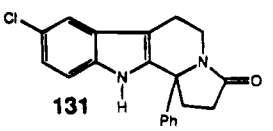
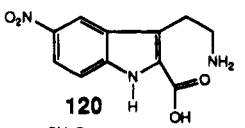
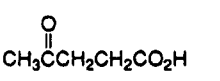
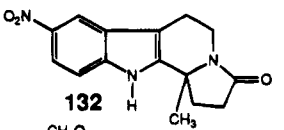
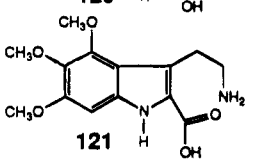
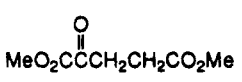
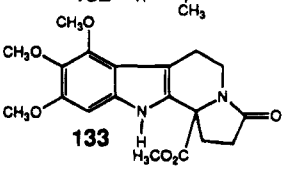
Mechanistically, the iminium ion **109** can undergo attack at C-3 to provide the spiroindolenine intermediate[5,56] **110** or the ion **109** can undergo direct attack at C-2[80] to provide the carbocation **111**. The former intermediate is unlikely due to the localization of positive charge adjacent to the carbonyl group (see resonance structure **113**, Scheme 10). It is known that 6-alkoxy substituents facilitate attack at C-2[80] in the Pictet–Spengler reaction. If this were the case then resonance structure **114** might be expected to play a role in the stabilization of intermediate **111** in the condensation reaction described herein. From Table 8 it is clear that the treatment of 6-methoxytryptamine-2-carboxylic acid (**118**) with α-keto ester **123** provided a higher yield of tetrahydro-β-carboline than the 5-methoxy analog **117** in agreement with direct attack at C-2 for this process.

The synthesis of the cytotoxic antileukemic alkaloid 1-methoxycanthin-6-one **105** was completed by Hagen et al.[99] and is illustrated in Scheme 11. This synthetic study also resulted in a general method for the synthesis of 4-alkoxy-β-carbolines from 4-oxo-1,2,3,4-tetrahydro-β-carbolines. The treatment of tryptamine hydrochloride **3** with the dimethyl ester of 2-ketoglutaric acid (**123**) in methanol at reflux provided the desired indolizino[8,7-*b*]indole lactam **134** in 92% yield; this same condensation occurs readily between **115** and **123** as well. During this process a Pictet–Spengler cyclization had occurred and the γ-lactam **134** had formed in a one-pot reaction. The γ-lactam contained the necessary carbon atoms for the synthesis of **105**; moreover, both the N_b-nitrogen atom and C-1 carbon atom were protected from interaction with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ). The desired 3-acylindole **135** was obtained in good yield when the γ-lactam **134** was stirred with DDQ (1:2) in aqueous THF at room temperature. The 4-oxo-1,2,3,4-tetrahydro-β-carboline **135** was heated in HCl/HOAc, according to the procedure of Hobson,[75] to remove the ester protecting group which resulted in the formation of 3-acylindole **136** in 88% yield. The γ-lactam of **136** proved to be resistant to hydrolysis under a variety of conditions.[99,100]

In order to facilitate cleavage of the γ-lactam to provide the δ-lactam, it was decided to form the enol ether of the 3-acyl indole **136**. This would provide a 1,2-dihydro-β-carboline, congeners of which are known to readily undergo oxidation (O_2, air) or disproportionation to provide the fully aromatic β-carbolines.[54,101] With this in mind, the keto lactam **136** was heated with trimethyl orthoformate in methanol in the presence of *p*TSA to provide a 51% yield of 4-methoxy-1-(3-carbomethoxypropyl)-β-carboline **137**. The ester group of **137** was hydrolyzed in quantitative yield with aqueous ammonia, followed by heating of the residual solid in the presence of *p*TSA to furnish 1-methoxy-4,5-dihydrocanthin-6-one (**138**) in 82% yield. Dehydrogenation of the δ-lactam **138** was accomplished using DDQ in dioxane at reflux to provide 1-methoxycanthin-6-one (**105**) in 70% yield. This seven-step synthesis of **105** proceeded in an overall yield of 20% starting from tryptamine **3** and dimethyl 2-ketoglutarate (**123**). This represented the first synthesis of any of the 1-methoxycanthin-6-one alkaloids and provides a route to other alkaloids in this series.

The carboxyl-mediated Pictet–Spengler reaction is an effective method for the direct synthesis of 1,2,3,4-tetrahydro-β-carbolines and hexahydro-3-oxo-indolizino[8,7-*b*]indoles from tryptamine-2-carboxylic acids. It is no longer necessary to remove the 2-carboxylic acid function to provide tryptamines prior to the

Table 8. Synthesis of Indolizino[8,7-*b*]indoles from Tryptamine-2-carboxylic Acids

tryptamine-2-acid	carbonyl component	product	(% yield)
 **115**	 **18**	 **125**	(75)
 **115**	 **34**	 **126**	(58)
 **115**	 **122**	 **127**	(85)
 **116**	 **123**	 **128**	(65)
 **117**	 **123**	 **129**	(60)
 **118**	 **123**	 **130**	(80)
 **119**	 **124**	 **131**	(60)
 **120**	 **122**	 **132**	(52)
 **121**	 **123**	 **133**	(66)

execution of the Pictet–Spengler reaction for the elements of CO_2 are lost during the process of cyclization. This greatly enhances the use of the Abramovitch–Shapiro method[94,95] for the synthesis of substituted-β-carbolines especially in the area of highly oxygenated ring A-substituted heterocycles. This method can be potentially extended to other indoles which contain a carboxyl function located at position-2. Heterocycles such as indole 2-esters are readily available through the Fischer,[102] Reissert,[103] and Moody[104,105] routes.

D. Kinetic vs Thermodynamic Control

Previous reports highlighted the variance in the diastereochemical product ratios obtained when employing the Pictet–Spengler condensation.[54,63,77] Three such examples are shown in Table 9. The ratios of diastereomers when acetaldehyde (**157**), propionaldehyde (**68**), and cyclohexanecarboxaldehyde (**23**) were heated with N_b-H tryptophan methyl ester under acidic conditions reportedly varied from 75:25 *cis/trans* (**139/140**) to a more equal ratio of 43:57 *cis/trans* (**64a/64b**), to 41:59 *cis/trans* (**25a/25b**). The only significant difference between these reactions was the size of the substituent at C-1 of the tetrahydro-β-carboline. We have noted previously that as the size of the substituent at C-1 increases, so too does the degree of *trans* stereoselectivity.[54,77] To examine the effect the steric bulk of the incoming aldehyde had upon the diastereomeric ratio realized in the Pictet–Spengler condensation in the presence of a benzyl group at the amino nitrogen, N_b-benzyltryptophan methyl ester was prepared.[52,106] This base was treated individually with acetaldehyde,

Scheme 11

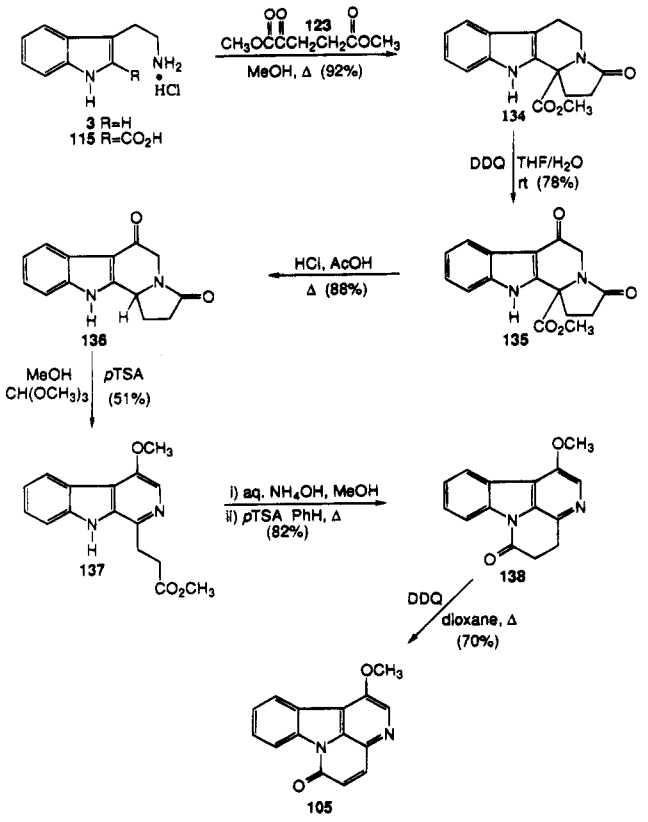
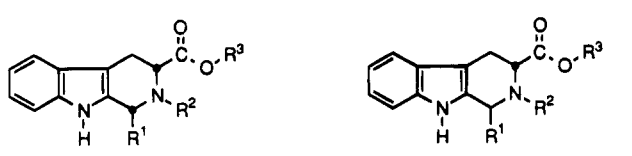


Table 9. *Cis:Trans* Ratios[a] of (±)-1,2-Disubstituted and 1,2,3-Trisubstituted Tetrahydro-β-carbolines



compound	R^1	R^2	R^3	aprotic *cis:trans*	acidic *cis:trans*
139, 140[b]	CH_3	H	CH_3		75:25
64a, 64b	CH_2CH_3	H	CH_3		43:57[c]
25a, 25b	C_6H_{11}	H	CH_3	40:60[c]	41:59[c]
141, 142	CH_3	Bn	CH_3	26:74	12:88
143, 144[d]	$CH_2CH_2CH_3$	Bn	CH_3	23:77	11:89
27	C_6H_{11}	Bn	CH_3	0:100	0:100
145, 146	CH_3	Bn	$CH(CH_3)_2$	23:77	13:87
147, 148	$CH_2CH_2CH_3$	Bn	$CH(CH_3)_2$	13:87	12:88
149	C_6H_{11}	Bn	$CH(CH_3)_2$	0:100	0:100
150, 151	CH_3	$CH(Ph)_2$	CH_3	10:90	0:100
152	$CH_2CH_2CH_3$	$CH(Ph)_2$	CH_3	0:100	0:100
153	C_6H_{11}	$CH(Ph)_2$	CH_3	0:0[e]	0:100
154	CH_3	$CH(Ph)_2$	$CH(CH_3)_2$	0:100	0:100
155	$CH_2CH_2CH_3$	$CH(Ph)_2$	$CH(CH_3)_2$	0:0[e]	0:100
156	C_6H_{11}	$CH(Ph)_2$	$CH(CH_3)_2$	0:0[e]	0:100

[a] As determined by integration of the ^1H NMR spectrum (±3%). [b] When a pair of numbers is present, the first is *cis*, the second *trans*. When only one number is present the reaction was 100% *trans* stereoselective. [c] Reference 77. [d] Carried out in the optically active series. [e] These transformations yielded only starting materials under nonacidic aprotic conditions but were converted into the 1-substituted tetrahydro-β-carbolines upon addition of TFA to the reaction medium.

butyraldehyde, and cyclohexanecarboxyaldehyde under the nonacidic aprotic conditions of Sandrin et al.[46,47,85] This afforded a series of 1-alkyl-2-benzyl-3-(methoxycarbonyl)tetrahydro-β-carbolines with the substituent at position 1 varying in steric bulk from the relatively small methyl group, to propyl, to the larger cyclohexyl moiety.

The reactants were heated in benzene at reflux under nonacidic aprotic conditions for 36 h. All reactions were stopped after 36 h to provide uniform experimental conditions, thus enabling an unbiased comparison of the stereochemical effects of the various substituents. Chemical yields are thus unoptimized. The reactions utilizing acetaldehyde were carried out in a sealed glass tube to avoid loss of the aldehyde. Each aldehyde was purified by vacuum distillation and the IR spectrum obtained to verify that the reactants were indeed free of acid. In all cases, after 36 h a small aliquot was removed and the proton NMR spectrum recorded. It was difficult to accurately measure the diastereomeric ratio due to impurities which masked the signals necessary to determine the ratio of diastereomers which were formed. In these cases impurities were removed by running the reaction mixture through a short wash column of silica gel and measuring the NMR spectrum once again. Comparison of the spectra before and after chromatography assured that the diastereochemical ratio had not been altered. Flash chromatography on silica gel permitted separation of the *cis* and *trans* diastereomers and a close examination of the NMR spectra of the individual diastereomers clearly indicated the proton signals that differed significantly. These signals were then used to determine the diastereomeric ratio of *cis* to *trans* isomers in the spectra of the mixtures. Although $CDCl_3$ was initially used as an NMR solvent it was found that the acidic impurities present were in high enough concentration to effect epimerization of the *cis* diastereomers to their *trans* isomers. The cause of this epimerization will be expanded upon in a later section. These impurities could be removed but it was found easier to employ C_6D_6 as the NMR solvent. Catalytic transfer hydrogenation of the 1,2,3-trisubstituted species afforded 1,3-disubstituted tetrahydro-β-carbolines that are well-known compounds.[54,77] Consequently, the stereochemistry of the individual diastereomers could be unequivocally established by comparison of their ^{13}C NMR spectra to previously published work. Determination of the stereochemistry of these individual diastereomers had been carried out according to the carbon NMR method described earlier, a method which has been validated by independent sources.[76-79]

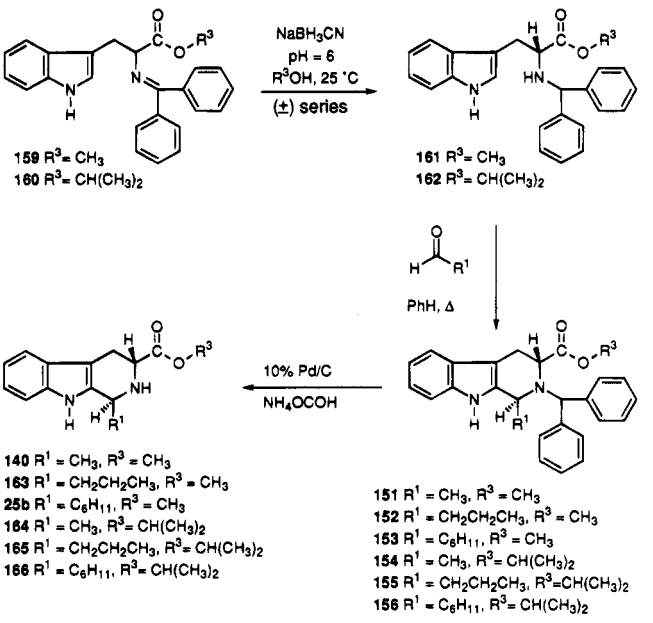
The condensation of N_b-benzyltryptophan methyl ester (**21**) with acetaldehyde (**157**) or butyraldehyde (**158**) resulted in the formation of mixtures of *cis* and *trans* diastereomers **141−144**, respectively. This same condensation employing cyclohexanecarboxaldehyde **23** provided only the *trans* diastereomer **27** as was expected.[60] Examination of the product ratios (Table 9) for the nonacidic aprotic condensations with acetaldehyde (26:74 *cis*-**141**/*trans*-**142**) and butyraldehyde (23:77 *cis*-**143**/*trans*-**144**) revealed that the ratio of *cis* to *trans* diastereomers for each was essentially the same. As the steric bulk of the substituents increased to cyclohexyl, however, the *cis* isomer was excluded from formation. Ungemach had proposed that this phenomenon was the result of a steric interaction between the ester function and the alkyl group in addition to the effect of $A^{(1,2)}$ strain.[60]

Since aldehydes similar in size to an *n*-butyl group provided a mixture of diastereomers the synthetic potential of the process was limited. If Ungemach's hypothesis were correct, however, any increase in the steric interactions between the ester and alkyl groups should shift the diastereomeric ratios toward increased *trans* stereoselectivity. To this end, replacement of the methyl ester function with the larger isopropyl group was undertaken. After the N_b-benzyltryptophan isopropyl ester was synthesized in a fashion analogous to the methyl ester, the previously mentioned series of condensations were repeated and the diastereomeric ratios examined by NMR spectroscopy. In agreement with this hypothesis one of the condensations yielded an increased amount of *trans* isomer, the condensation of N_b-benzyltryptophan isopropyl ester with butyraldehyde (**158**). In the 1-propyl methyl ester series (**143** and **144**) the ratio of *cis* to *trans* was 23:77 for this condensation while in the isopropyl ester series (**147** and **148**) the amount of *trans* isomer was increased to 13:87 (*cis*:*trans*).

The previous work in this area had clearly demonstrated that introduction of a benzyl group at the N_b-amino nitrogen function favored formation of the *trans* diastereomer. Substituents at the amino N_b-nitrogen function have come under scrutiny previously since it was shown by Ottenheijm et al.[82] and Sandrin et al.[85] that electronic effects played a significant role in the stereochemical outcome of the reaction. The effect of the steric bulk of the N_b-alkyl substituent upon the diastereochemical ratio had not been fully studied. In order to increase the size but not alter the electronic effects on the stereochemical preference of this condensation, the substituent at the N_b-nitrogen function, a diphenylmethyl group, was employed. Introduction of this group (Scheme 12) was accomplished by transimination of the appropriate ester hydrobromide or hydrochloride salt with benzophenone imine, according to the procedure of Polt and O'Donnell.[107] Reduction of the resultant imine with sodium cyanoborohydride in methanol or 2-propanol under acidic conditions provided the desired N_b-diphenylmethyl-substituted tryptophan derivative **161** or **162**, respectively. This facile reduction could be carried out in as little as 5 min (depending upon the scale of the reaction) with complete conversion of the imine. This protocol provided excellent yields, could easily be scaled up to the 10 g level, and was not subject to disubstitution or the formation of quaternary ammonium salts. With the methyl and isopropyl esters of N_b-diphenylmethyltryptophan in hand, the series of condensations were repeated (benzene at reflux) and the diastereomeric ratios measured by NMR spectroscopy.

In the methyl ester series the condensation with acetaldehyde reacted to form the desired 1,2,3,4-tetrahydro-β-carbolines (**150** and **151**) as evidenced by ^{1}H NMR spectroscopy (10:90) after initial removal of the nonindolic byproducts. However, attempts to isolate the small amount of *cis* diastereomer which was present were unsuccessful. Only the *trans* diastereomer **151** was found upon examination of the chromatographic fractions. In the reaction of N_b-(diphenylmethyl)tryptophan isopropyl ester (**162**) with acetaldehyde, only the *trans* diastereomer **154**

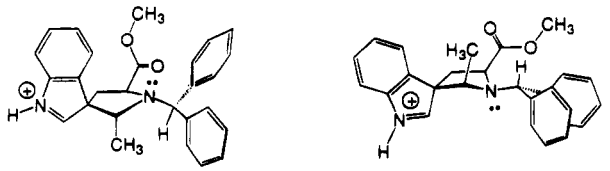
Scheme 12



was formed as determined by ^{1}H and ^{13}C NMR spectroscopy. In the methyl ester series the reaction of butyraldehyde also provided only the *trans* isomer **152**. In three of the cases (**153**, **155**, and **156**) no reaction was observed under nonacidic aprotic conditions after 36 h presumably due to increased crowding in the transition states. Prolonged heating only increased the amount of byproducts which were formed. In order to effect the Pictet–Spengler cyclization it was necessary to add trifluoroacetic acid (TFA) to the reaction medium. The addition of TFA to catalyze the formation of **153**, **155**, and **156** resulted in the isolation of only the *trans* diastereomer in each case. Cleavage occasionally of diphenylmethyl groups is known to occur with TFA at low concentrations,[108–110] although it failed to do so here as evidenced by control reactions devoid of aldehyde. In all the reactions employed with the N_b-diphenylmethyl group, the chemical yields are somewhat lower than in the N_b-benzyl cases. This is not unreasonable since the presence of such bulky moieties hinders the formation of 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines.[62,108–110]

A comparison of the reactions which employed acetaldehyde showed a marked trend. In the N_b-H methyl ester case (**139** and **140**, 75:25 *cis*/*trans*), the Pictet–Spengler reaction yielded the *cis* isomer as the major product. Introduction of a benzyl function at the amino nitrogen atom inverted the stereochemical outcome to provide the *trans* diastereomer under aprotic conditions (**141** and **142**, 26:74 *cis*/*trans*) as the major product. Furthermore, when the N_b substituent was increased to the larger diphenylmethyl group under similar conditions, a 9:1 stereoselective formation of the *trans* diastereomer **151** was observed (Table 9). When the methyl ester substituent was replaced with an isopropyl group a 100% stereoselective formation of the *trans* diastereomer **154** was realized.

As previously shown in Scheme 9, two distinct spiroindolenine intermediates may result from attack on the imine intermediate. In the N_b-diphenylmeth-



167a *anti* spiroindolenine intermediate

167b *syn* spiroindolenine intermediate

Figure 5. *Syn*- and *anti*-spiroindolenine intermediates.

yltryptophan methyl ester case, attack of the imine from the face opposite the ester function would provide the *anti* spiroindolenine **167a**. Attack from the same face as that occupied by the ester would result in a spiroindolenine of *syn* configuration such as intermediate **167b**, as illustrated in Figure 5. Molecular mechanics calculations combined with conformational searching (MacroModel 2.5–MM2 force field) revealed that the *anti* configuration is 2.1 kcal/mol lower in energy than the all eclipsed *syn* configuration.[62] On rearrangement to C-2 this *anti* spiroindolenine **167a** can only provide the *trans* diastereomer.

The data from these experiments under nonacidic aprotic conditions (benzene) reflect ratios which are the result of kinetic trapping experiments. Examination of the data in Table 9 clearly indicated that if mixtures of N_b-alkyl diastereomers realized in the nonacidic aprotic Pictet–Spengler reaction were exposed to acid, the diastereomeric ratios in the N_b-alkyl cases shifted to further increase the amount of *trans* diastereomer formed. This generality was confirmed by either the addition of TFA to a small aliquot of the reaction mixture or as in cases with tetrahydro-β-carbolines **153**, **155**, and **156**, by reaction in the presence of TFA to catalyze cyclization. Examination of ratios **141/142**, **143/144**, **145/146**, and **150/151**, clearly indicated that the ratio of *cis* product to *trans* product formed under nonacidic aprotic conditions was higher than that realized under acidic conditions. The method by which the conversion of the *cis* diastereomers into the more thermodynamically stable *trans* diastereomers took place can now be rationalized.

E. Epimerization at C-1 by Scission of the Carbon–Nitrogen Bond (Thermodynamic Control)

For these studies optically active N_a-methyl-N_b-benzyl-D-(+)-tryptophan methyl ester was synthesized from D-(+)-tryptophan (**168**, Scheme 13). Alkylation of D-(+)-tryptophan with methyl iodide in sodium–liquid ammonia at −78 °C and esterification of the resultant solid by heating in methanolic hydrogen chloride at reflux provided ester **169**. This indole was converted into N_a-methyl-N_b-benzyltryptophan methyl ester (**170**) on stirring the free base with benzaldehyde at 18 °C for 2 h, followed by reduction of the imine with sodium borohydride at −5 °C. Racemization was observed if the benzylation process took too long or the temperature rose too high,[111] although in the racemic series (17 h) it made no difference. The optical purity of the above compounds was verified by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent tris[3-[(trifluoromethyl)hydroxymethylene]-(+)-camphorato]europium(III) and later by HPLC analysis of diastereomeric ureas.[24,106,112]

Scheme 13

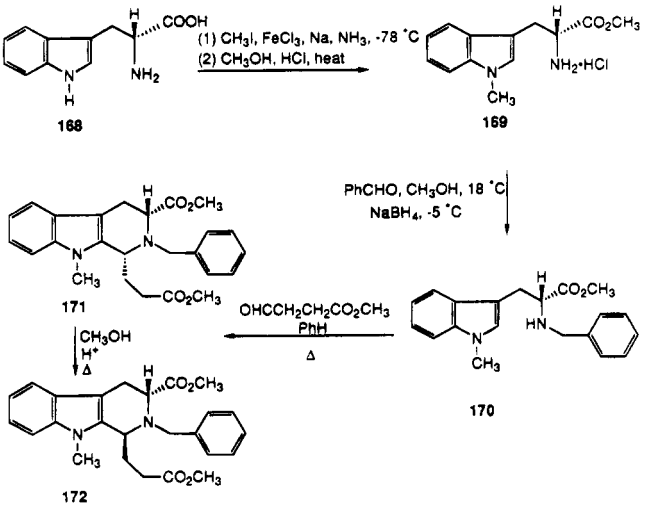
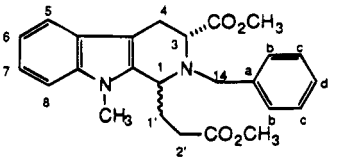


Table 10. ^{1}H NMR Data for 171 and 172



proton	**172** (*trans*) chemical shift δ (ppm)	**172** (*trans*) *J* (Hz), multiplicity	**171** (*cis*) chemical shift δ (ppm)	**171** (*cis*) *J* (Hz), multiplicity
H (1)	3.51	15.7/5.2, dd	3.75	9.5, d
H (3)	4.10	11.0/5.5, dd	3.92	6.3/2.1, dd
H (4_a)	3.05	15.8/5.5, dd	3.05	18.6/6.3, dd
H (4_b)	3.12	15.8/11.0, dd	3.37	18.6/2.1, dd
H (5)	7.60	8.0, d	7.58	9.5, d
CH_3 (N_9)	3.84	s	3.69	s
CH_3 (ester)	3.65	s	3.65	s
CH_3 (ester)	3.50	s	3.56	s
H_8 (aromatic)	7.12–7.40	m	7.10–7.48	m
H (14_a)	3.39	13.1, d	3.89	s (14_a/14_b)
H (14_b)	3.81	13.1, d		
H ($1'_a$)	1.85	m	1.50	m
H ($1'_b$)	2.02	m	1.95	m
H ($2'_a$)	2.38	17.5/5.6, dt	2.51	18.0/6.0, dt
H ($2'_b$)	2.61	17.5,9.6/5.6, d, dd	2.81	18.0,9.8/6.0, d, dd

Reaction of optically active N_a-methyl-N_b-benzyltryptophan methyl ester **170** with methyl 3-formylpropionate in benzene at reflux provided a mixture of the optically active diastereomers **171** $[\alpha]^{25}_D$ = −36.8° (*c* = 0.95, $CHCl_3$), and **172** $[\alpha]^{25}_D$ = +20.0° (*c* = 0.96, $CHCl_3$) in a *trans/cis* ratio of 72:28 (81%). The *trans* isomer **172** (mp 119–120 °C) was identical spectrometrically to the (±)-*trans* isomer (mp 145–146 °C) whose structure had been confirmed by Yoneda by X-ray crystallographic analysis.[113] It was interesting to note that both the C-1 substituent and the N_b-benzyl function of the *trans* isomer occurred in the axial position in the solid state as confirmed by the X-ray analysis.[113] The coupling constants observed between H-3 and H-4a, H-4b (*J* = 5.5 and 11.0 Hz) indicate that H-3 was located on the β-axial position of the chair-like C-ring with a dihedral angle of 135° with respect to H-4a and 10° in regard to H-4b (Table 10). Hence the *trans* isomer **172** contained an axial substituent at C-1, an equatorial substituent at C-3 and an axial benzyl group in the preferred

Table 11. ^{13}C NMR Data for 171 and 172

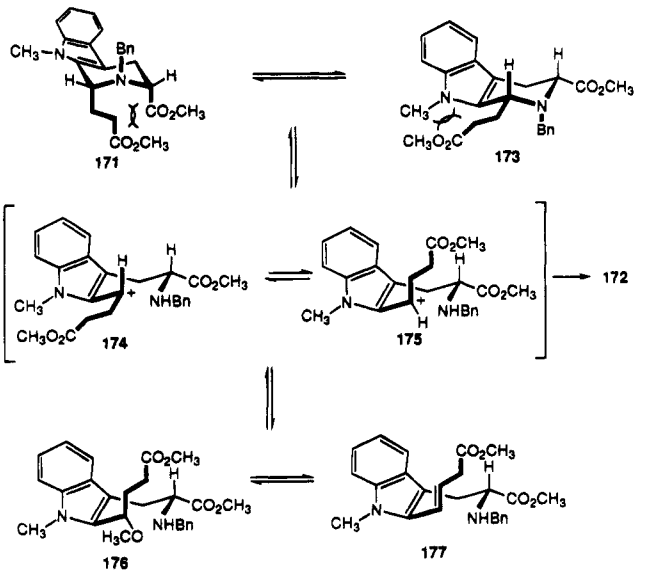
carbon atom	172	171	racemic[a]
C (1)	53.32	54.09	53.5
C (3)	56.12	57.44	56.2
C (4)	20.25	17.95	20.4
C (5)	118.12	118.25	118.2
C (6)	119.11	118.93	119.2
C (7)	121.32	121.28	121.4
C (8)	108.90	108.79	108.9
C (10)	135.67	134.65	135.7
C (11)	106.29	104.70	106.4
C (12)	126.52	126.58	126.7
C (13)	137.46	137.49	137.6
C (14)	52.79	61.16	52.9
C (14a)	139.25	138.93	139.2
C (14b)	129.29	129.07	129.3
C (14c)	128.14	128.36	128.2
C (14d)	126.96	127.31	127.0
C (1′)	27.90	29.10	28.0
C (2′)	29.60	29.66	29.68
N-CH_3	29.71	29.66	29.74
$COOCH_3$	51.26	51.30	51.3
$COOCH_3$	52.00	51.83	52.0
C=O	173.37	174.07	173.3
C=O	173.87	174.25	173.9

[a] Reference 118.

conformation in solution. This was further confirmed by ^{13}C NMR spectroscopy. In the ^{13}C NMR spectrum of the two diastereomeric 1,2,3,4-tetrahydro-β-carbolines, it was well documented that the exocyclic benzylic methylene carbon atom (C-14) of the N_b-benzyl-*cis*-1,3-disubstituted-1,2,3,4-tetrahydro-β-carboline resonated downfield from that of the corresponding *trans* isomer.[68,114] As illustrated in Table 11, the chemical shift of carbon atom 14 in the *cis* isomer **171** (δ 61.16) appeared 8.37 ppm downfield with respect to the corresponding carbon atom in the *trans* diastereomer **172** (δ 52.79). This phenomenon was due to the γ-gauche effect.[68,77,114] The preferred position of the N_b-benzyl group in the *cis* isomer **171** must be *trans* to the two 1,3-substituents, while in the corresponding *trans* isomer, any position occupied by the benzyl group will necessarily be *cis* either to the substituent at C-1 or C-3. Consequently, this carbon atom (C-14) is compressed in the ^{13}C NMR spectrum of *trans* isomer **172**, and resonates up field with respect to the corresponding carbon atom in the *cis* isomer **171**. The coupling constants between H-3 and H-4a, H-4b (J = 6.3 and 2.1 Hz) indicate that hydrogen atom H-3 of the *cis* isomer **171** was located in the α, equatorial position with a dihedral angle of 35° with respect to H-4a and 85° with regard to H-4b (Table 10). The benzyl group in the *cis* diastereomer should be in the β, axial position in order to reduce the steric repulsion with the *cis* 1,3-substituents. This was confirmed by the ^{13}C NMR spectrum as described previously on the N_b-H analogs.[5] The acids corresponding to the *trans* **172** and *cis* **171** diastereomers were also obtained (58:42) in optically active form when the Pictet–Spengler reaction of **170** was executed with α-ketoglutaric acid (84%) in benzene–dioxane.[111] The mechanism of diastereoselectivity in the Pictet–Spengler condensation of α-keto acids is different from the mechanism which has been discussed thus far.[1,111]

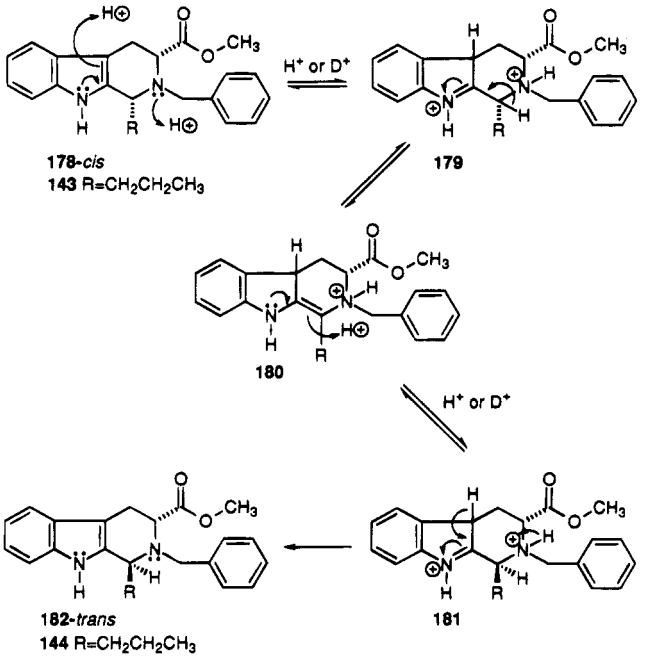
There were a number of reports in the literature regarding the synthesis of analogs of the *cis* isomer

Scheme 14

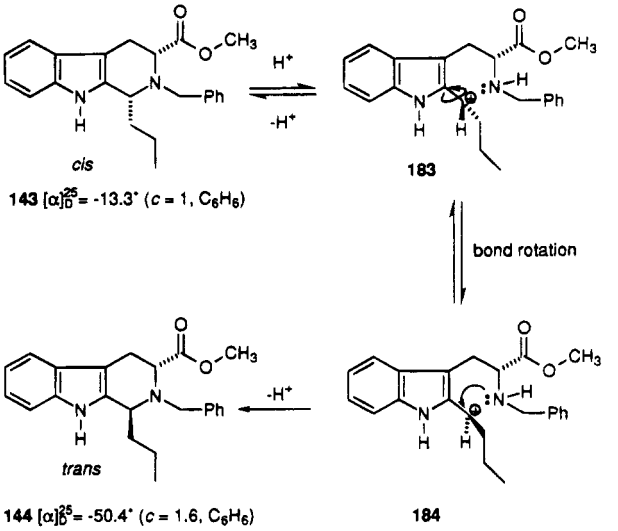


171.[45,113,115–120] However, little was known about the effect of the substituents located 1,3 on the tetrahydro-β-carboline ring with regard to inversion of configuration in these molecules. It was observed that the *cis* isomer (+)-**171**, could be converted into the thermodynamically more stable *trans* diastereomer (−)-**172** (85%), $[\alpha]^{25}_D$ = −36.6° (*c* = 1.0, CH_3Cl), by heating *cis* isomer **171** in a 1% anhydrous HCl–methanol solution at reflux. The optically active *trans* isomer **172** remained unaffected when treated under the analogous conditions. The epimerization must have occurred at C-1 of the *cis* diastereomer since only optically pure *trans* ester **172** was isolated from the reaction. If epimerization of the *cis* diastereomer had occurred at the C-3 carbon atom (ester) of **171**, it would have resulted in the formation of the enantiomer of **172** with an expected specific rotation ($[\alpha]^{25}_D$ = +36°) equal and opposite to that of **172**. This was contrary to the observed results. Further evidence for the epimerization of *cis* **171** at C-1 to provide *trans* **172** was obtained on isolation of intermediates **176** and **177** (Scheme 14). Both the methyl ether **176** and alkene **177** were minor products (7%) obtained from the acid promoted conversion of *cis* **171** into *trans* **172**. The structures of **176** and **177** were determined on the mixture by COSY NMR and mass spectroscopy.[111] When a mixture of intermediates **176** and **177** was heated in methanolic HCl (1%), the *trans* diastereomer **172** was isolated as the sole product (70%), $[\alpha]^{25}_D$ = −36.6° (*c* = 1.0, $CHCl_3$). None of the *cis* isomer **171** was observed. On the basis of the above experiments, the epimerization of (1*R*,3*R*)-(+)-*cis*-**171** to provide (1*S*,3*R*)-(−)-*trans*-**172** had occurred regiospecifically at C-1 and could be rationalized, as illustrated in Scheme 14. Under the conditions of heat and acid the N_b-nitrogen atom of *cis* isomer **171** can be protonated, followed by ring cleavage across the 1,2(C–N) bond to furnish the carbocations **174** or **175**. The carbocation **174** (or **175**) may either react with methanol to give 1-substituted ether **176** or lose a proton to furnish alkene **177**. These carbocations, **174** or **175**, which occupy a central position in the equilibrium, may also cyclize via a sterically favored conformation **175** to furnish

Scheme 15. Alternate Mechanism for the *Cis* to *Trans* Epimerization at C-1 Does Not Operate Here



Scheme 16. Proposed Mechanism for the Acid-Catalyzed Epimerization of the *Cis* to *Trans* Tetrahydro-β-carboline



the optically active *trans* diastereomer **172**. Presumably, an equilibrium between the *cis* 1,3-diaxial **171** and *cis* 1,3-diequatorial **173** conformers existed at high temperature. The driving force for the ring scission between C-1 and N-2 was presumed to result from relief of $A^{(1,2)}$ strain[69,121] between substituents located at C-1 and N-9 in the diequatorial conformer **173** or the 1,3-diaxial interactions between substituents at C-1 and C-3 in the corresponding diaxial conformer **171**.

Another possible mechanism for this *cis*–*trans* epimerization involved the initial protonation of the indole 3-position and subsequent formation of an olefinic species (Scheme 15). This olefin could then be protonated from either face of the double bond to yield the more thermodynamically stable *trans* diastereomer **182**. Although Zhang provided strong evidence with α-ketoglutaric acid that this olefinic equilibration was not involved,[111] recent results have further confirmed this earlier work.

In order to test the model for carbon–nitrogen bond cleavage versus the olefinic protonation model in the absence of an N_a-methyl function, two experiments were carried out. In the first, optically active N_b-benzyltryptophan methyl ester was synthesized from D-(+)-tryptophan according to the method employed for the related N_a-methyl compound, as described above. This material was stirred under the nonacidic aprotic conditions of benzene at reflux under a nitrogen atmosphere with freshly distilled butyraldehyde **158** to kinetically trap the optically active diastereomers as a 23:77 *cis/trans* mixture. Separation of the diastereomers on silica gel with CH_2Cl_2 by gravity chromatography provided the pure *cis* and *trans* diastereomers **143** and **144**. The specific rotation $[\alpha]^{25}_D$ of the *trans* diastereomer **144** was measured as −50.4° (c = 1, benzene) and the *cis* **143** as $[\alpha]^{25}_D$ = −13.3° (c = 1, benzene). The (−)-*cis* diastereomer **143** was then taken up in CH_2Cl_2 and stirred with 2 equiv of TFA. Examination of the mixture by TLC after 12 h revealed that the *cis* isomer had been completely converted into the *trans* diastereomer **144** (Scheme 16). Workup with mild base ($NaHCO_3$) and chromatography on a short wash column of silica gel (CH_2Cl_2) yielded the (−)-*trans* enantiomer **144**. The specific rotation $[\alpha]^{25}_D$ of this sample was −50.4° (c = 1.6, benzene). Since the specific rotations of the two (−)-*trans* compounds were identical (as well as the NMR and mass spectra) the epimerization that Zhang observed across the C(1)–N(2) bond had occurred here as well.

To test the alternate mechanism of *cis*–*trans* epimerization, the same experiment was repeated with 2 molar equiv of pure CF_3COOD (Scheme 15). If the olefin protonation mechanism had operated, then deuterium would have been incorporated into the tetrahydro-β-carboline at C-1 upon protonation of the olefin **180** to re-form the *trans* tetrahydro-β-carboline **144**. Repke et al. in an ibogaine derivative, have shown that incorporation of deuterium at position 1 was found to occur when an olefinic mechanism of isomerization was in operation.[122] We found no evidence for deuterium incorporation at C-1 of **144** by integration of the ^1H NMR spectrum nor by mass spectroscopy.

It was clear that the isomerization of the *cis* diastereomers to the more thermodynamically stable *trans* diastereomers (in both cases—Scheme 14 and Scheme 16) did not take place by the olefinic intermediate **180** (Scheme 15), but presumably by cleavage across the C(1)–N(2) bond (Schemes 14 and 16). In the experiments which involve N_a-H derivatives **143** and **144**, it was observed that upon treatment of the *cis* isomer **143** with acid, **143** was completely converted into the *trans* diastereomer **144**, as illustrated in Scheme 16. After examination of the reaction mixture by TLC, *cis* isomer **143** was not observed and the reaction mixture was then heated to reflux and followed hourly by TLC. After prolonged heating no *cis* isomer (**143**) was observed in the mixture. Furthermore, there was no evidence of the original starting material (N_a-hydro-N_b-benzyl-

tryptophan methyl ester) which would be indicative of a retro-Pictet–Spengler-type process. From these experiments it has been concluded that the *trans* diasteromers are thermodynamically more stable than their *cis* counterparts in the N_a-hydro-N_b-alkyl series as well. Hence, exclusive formation of the *trans* isomer would occur if the *cis* diastereomer were allowed to react under acidic conditions. Massiot and Mulamba[123] reported that a *trans* tetrahydro-β-carboline was formed exclusively when methyl 4-formyl-2,2-bis(phenylthio)butyrate and tryptophanamide were heated to form an imine after which cyclization was effected with TFA at room temperature in CH_2Cl_2. On the basis of the results under aprotic conditions which yielded diastereomeric mixtures (Table 9), it seems more reasonable that a mixture of the *cis* and *trans* diastereomers had been formed by Massiot et al. and that upon introduction of TFA the *cis* diastereomer had epimerized to the more thermodynamically stable *trans* diastereomer. When optically active N_b-benzyl-D-(+)-tryptophan methyl ester and butyraldehyde were again heated in benzene at reflux until TLC indicated the consumption of all the indolic starting material, it was found that the *trans* diastereomer **144** could exclusively be formed upon addition of 10 equiv of TFA at room temperature to the reaction mixture. The epimerization of the *cis* diastereomer **143** to the *trans* diastereomer **144** was monitored by TLC (74% yield). This has important implications for the enantiospecific synthesis of indole alkaloids. The use of N_b-benzyl or N_b-diphenylmethyl substituents in the tryptophan methyl ester series can provide the *trans* diastereomer kinetically with 100% diastereoselectivity. In cases where a mixture of the *cis* and *trans* diastereomers are formed, the thermodynamic preference for the *trans* isomer in the N_b-alkyl series can be employed to convert the entire mixture into the desired *trans* isomer with 100% diastereoselectivity. Moreover, if the Pictet–Spengler reaction is carried out in benzene at reflux followed by the addition of TFA afterward, this will provide the *trans* isomer stereospecifically (substituent at C-1 greater than CH_3). Since both D-(+)- and L-(−)-tryptophan are commercially available, both optical antipodes of the alkaloids are available enantiospecifically. Furthermore, the realization of the recent enantiospecific synthesis of both 5-methoxy- and 6-methoxy-D-(+)- or L-(−)-tryptophans provides enantiospecific entry into over 70 different alkaloids via this method.

III. Modeling the Pharmacophore of Benzodiazepine Receptor Sites

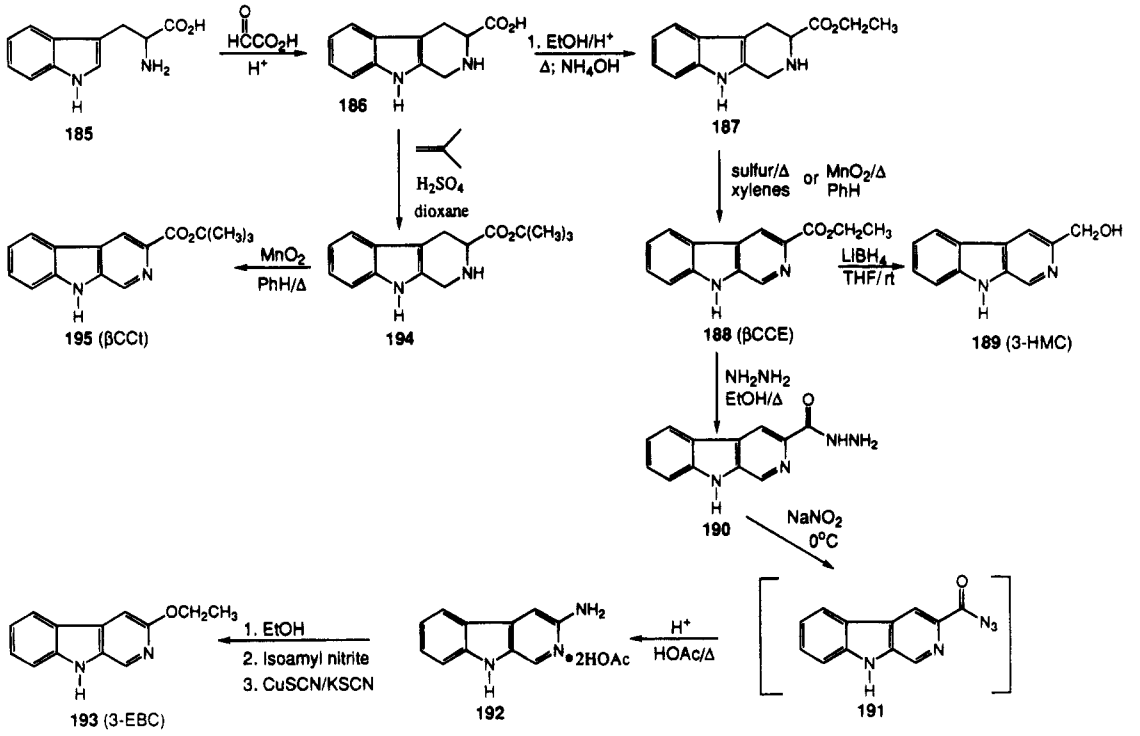
The study of benzodiazepine receptors has been one of the most rapidly increasing areas of research in molecular neuropsychopharmacology in recent years.[124–127] Benzodiazepines exhibit a wide range of clinical uses including the treatment of anxiety and related emotional disorders as well as sleep disorders and as anticonvulsants. They behave as centrally acting muscle relaxants. In 1975 examination of evidence from behavioral, electrophysiological, and biochemical experiments, indicated that benzodiazepines act at synapses in which γ-aminobutyric acid (GABA) is the acting neurotransmitter.[128] In 1977 the identification of specific, saturable, high-affinity binding sites for tritiated diazepam by two independent research groups, Möhler and Okada,[129] as well as Squires and Braestrup,[130] generated a tremendous interest in benzodiazepine receptor (BzR) sites. It was later determined through various biochemical techniques that the BzR comprises a portion of the $GABA_A$ receptor chloride ion complex. In addition to the benzodiazepine binding site, there exists at least three other modulatory binding domains on the complex corresponding to sites for barbiturates, picrotoxin, and anesthetic steroids.[126]

More recently, the $GABA_A$ receptor has been shown to be a heterooligomeric family of ligand-gated ion channels which constitutes the major inhibitory neurotransmitter system in the central nervous system (CNS).[125,126,131–134] This membrane-bound protein complex plays a central role in the molecular mechanisms which underlie anxiety, sleep, convulsions, memory-learning, and consequently it represents an important target for the design of selective agents to treat specific disease states in the CNS.[131–139] The inhibitory GABA neurotransmitter or ligand, acts by binding to its receptor site which is followed by the opening of an intrinsic chloride ion channel. The increase in chloride ion flux results in hyperpolarization of the neuronal cell membrane with a concomitant decrease in neuronal transmission. The biological activity of modulatory BzR ligands of interest here encompasses a wide range of physiological responses ranging from full agonist (anxiolytic, muscle relaxant/ataxic, amnesic, sedative/hypnotic, and anticonvulsant) to full inverse agonist (anxiogenic, proconvulsant, and convulsant). Clinically, there is a need for partial agonists which exhibit anxiolytic/anticonvulsant activity in the absence of myorelaxant, ataxic, and sedative-hypnotic activity. Moreover, there is a need for partial (or selective) inverse agonists which enhance neuronal firing in the CNS but are devoid of the proconvulsant/convulsant activity of full inverse agonists. These latter ligands would be useful for treatment of barbiturate–alcohol induced CNS depression (overdose), hepatic encephalopathy, and cognition enhancement among others.[137–139] Recent studies have shown that many β-carbolines do elicit these pharmacological properties.[44]

A. Molecular Biology

In 1987 Seeburg, Schofield, and co-workers reported the cloning and functional expression of a GABA/Bz receptor complex from bovine brain.[140] It was initially proposed the complex was composed of α- and β-subunits. Subsequent to this report, several other subunits of the $GABA_A$ receptor have been characterized including γ-, δ-, and ϱ-subunits.[127,141–143] The identification of multiple α-, β-, and γ-subunits is consistent with the pharmacological evidence of multiple $GABA_A$ receptor isoforms in the CNS.[144] Recent molecular biological studies have established that expression of at least two (α, γ) and preferably three (α, β, γ) subunits is necessary to constitute a functional receptor which mimics many of the pharmacological, biochemical, and electrophysiological properties of native receptors.[145–147] Previous studies of site-directed mutagenesis have shown that changes

Scheme 17



in the α-subunit altered the activity of the benzodiazepine receptor-mediated response.[37,138,148,149] It is now known that changes in the γ-subunit also alter this response.[147,150–152] However, neither the stoichiometry, nor the composition of native $GABA_A$ receptors in the CNS has been unequivocally established. It appears now, on the basis of preliminary evidence,[132,141,143,147,151,152] that the BzR binding site lies between the α- and γ-subunits.

B. Receptor Subtypes

Initially it was proposed that the type-I BzR was responsible for the anxiolytic and anticonvulsant effects[153] of the benzodiazepines while the type-II BzR mediated the muscle relaxant, sedative-hypnotic properties of these ligands. Structure–activity relationship (SAR) data from a variety of recently synthesized BzR ligands,[44,143,154–156] however, demonstrated that the actual pharmacological situation is much more complicated. Bz type-I selective ligands (i.e. CL 217872) have now been shown to exhibit some sedative effects,[154,157] in contrast to previous reports.[153] In fact zolpidem is now marketed as a sedative-hypnotic. Receptor isoforms whose pharmacology resembles that of previously reported Bz-I and Bz-II receptors have recently been expressed: the type-I BzR was constructed from an α1β2γ2 [α1β2γ2=ω1=α1] combination of subunits while the type-II BzR was comprised of α2β2γ2 [α2β2γ2=ω2=α2], α3β2γ2 [α3β2γ2=ω3=α3], and α5β2γ2 [α5β2γ2=ω5=α5] (zolpidem insensitive)[156] receptor isoforms. Moreover, photolabeling experiments[158] with [^{3}H]Ro 15-4513 have identified a site termed the "diazepam-insensitive" (DI) receptor which is made up of the subunits designated α4β2γ2 [α4β2γ2=ω4=α4] and α6β2γ2 [α6β2γ2=ω6=α6],[158,159] the latter of which has been studied extensively.[44] The physiological activity of agonists and inverse agonists at benzodiazepine receptors in the CNS is therefore a consequence of action at one or more of the receptor subtypes. Studies are currently underway to correlate receptor subtype selectivity with a biological response.

C. Biology of Selected 3-Substituted β-Carbolines

It was originally proposed by Braestrup that β-carboline-3-carboxylic acid ethyl ester (βCCE, **188**) shown in Scheme 17 was the endogenous ligand associated with the BzR.[160,161] Subsequent studies, however, have shown this ligand was formed during the isolation procedure.[124,162] Nonetheless, the demonstration that certain β-carbolines potently inhibit [^{3}H]diazepam binding with high affinity (5 nM) has led to numerous studies of ligands which bind to benzodiazepine receptors as well as the development of potential new therapeutic agents.

It has been shown that the full inverse agonist βCCE (**188**) antagonized the anticonvulsant actions of diazepam, lowered the seizure threshold of the convulsant pentylenetetrazole, and antagonized the sedative actions of flurazepam.[163–165] More importantly, when βCCE (**188**) was administered to primates[36] it elicited a profound behavioral and physiological syndrome reminiscent of "fear" or "anxiety" related to the same effects experienced in man. For example, in *Rhesus* monkeys this substance produced dramatic elevations in heart rate, blood pressure, plasma cortisol, and catecholamines.[36] These effects were blocked by benzodiazepines and the specific benzodiazepine receptor antagonist Ro 15-1788 (flumazenil). The results of this study demonstrated that the administration of βCCE (**188**) to animals may represent a reliable and reproducible model of human anxiety and as such, could be valuable in studying the postulated role of anxiety and stress in a variety of human diseases, including cardiovascu-

lar, ulcerative, and neoplastic disorders. In a beautiful series of experiments with βCCE (**188**) Mueller has shown that a BzR mechanism participates in the physiological regulation of β-endorphin-like immunoreactivity (β-END-LI) secretion from the AL of the rat pituitary gland. Accordingly, the ability of the anxiogenic β-carbolines βCCE (**188**) and βCCM (methyl ester) to stimulate rapid and pronounced increases in plasma β-END-LI indicates that anxiety, *per se*, is a stimulus for β-END-LI release. Furthermore, the findings raise the possibility that pituitary β-END peptides may have functions related specifically to anxiety and separate from pain.[166]

Although βCCE (**188**) is a potent inverse agonist, it has several serious drawbacks when used for *in vivo* studies. βCCE (**188**) is readily susceptible to esterase hydrolysis[43] to the inactive acid (especially in rodents), and thus large quantities of this compound are needed for pharmacological studies. In addition, βCCE (**188**) is only sparingly soluble in water, making administration of this compound difficult. Therefore, the search for water-soluble, long-lived partial inverse agonists led to the synthesis of 3-ethoxy-β-carboline (3-EBC, **193**), 3-(hydroxymethyl)-β-carboline (3-HMC, **189**) and β-carboline-3-carboxylic acid *tert*-butyl ester (βCCt, **195**), as shown in Scheme 17. The biology of these 3-substituted β-carbolines is discussed below.

Pharmacological studies on 3-HMC[167] (**189**) have shown this β-carboline inhibited [^{3}H]diazepam binding *in vitro* (K_i = 1470 nM), and antagonized both the anticonvulsant and anxiolytic actions of diazepam at doses which did not elicit overt behavioral effects. This β-carboline also inhibited the sleep-inducing effects of flurazepam[38] and was oxidized to the active aldehyde (IC_{50} = 62 nM) *in vivo*. Thus the hypnotic actions of flurazepam were strongly felt to originate through interaction with the benzodiazepine receptor. At slightly higher doses, 3-HMC (**189**) increased wakefulness in rodents by significantly increasing sleep latency and reducing non-REM (but not REM) sleep. Consequently 3-HMC (**189**) was not merely a benzodiazepine antagonist but exerted a pharmacological action opposite to that effected by 1,4-benzodiazepines. Although often drugs such as amphetamines and methylxanthines can reduce sleep, they also invariably cause profound alterations in behavior and motor activity. Compounds that reduce sleep without eliciting major changes in motor activity may, therefore, be more properly termed "somnolytics".[38] The suggestion (with 3-HMC, **189**) that the BzR was involved in both physiological and pharmacologically induced sleep[38] could lead to the development of β-carbolines or related compounds for treating human sleep disorders, especially those characterized by excessive somnolence.

3-Ethoxy-β-carboline (3-EBC, **193**) bound with high affinity to the BzR (IC_{50} = 24 nM). Trullas et al.[43] have shown that 3-EBC (**193**) potentiated the convulsant actions of pentylenetetrazole in mice consequently, it is proconvulsant. Furthermore, this compound reduced both the time spent and the total entries in the open arms of an elevated plus maze. Moreover 3-EBC (**193**) inhibited stress-induced ulcer formation in mice. These effects are common to benzodiazepine receptor inverse agonists, suggesting this compound is an inverse agonist at benzodiazepine receptors. Although 3-EBC (**193**) is proconvulsant, even at doses as high as 40 mg/kg it does not exhibit convulsant effects, consequently, it is termed a partial inverse agonist. In addition, this ligand has a higher affinity for benzodiazepine receptors and better water solubility (13 mg/mL of H_2O)[168] as a hydrochloride salt than the commonly employed inverse agonist FG 7142 (<1 mg/mL of H_2O), making this β-carboline an attractive ligand for *in vivo* studies in models of anxiety.

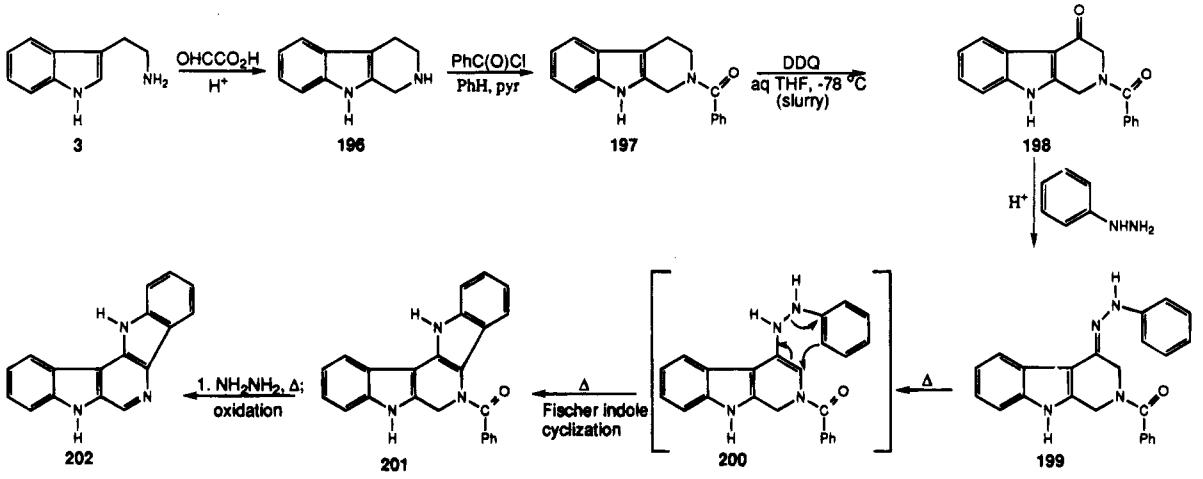
The β-carboline βCCt (**195**) was synthesized to provide a BzR ligand which would possess a longer duration of action than either ethyl ester βCCE (**188**) or methyl ester βCCM. This β-carboline **195** did bind tightly to BzR (IC_{50} = 10 nM) obtained from synaptosomal membranes and was much longer lived *in vivo* than the related ethyl or methyl esters.[39] The ability of βCCt (**195**) to antagonize the anticonvulsant, anxiolytic, ataxic, and muscle relaxant effects of diazepam (Valium) was evaluated by Shannon.[39] In mice βCCt (**195**) at doses of 3 and 10 mg/kg produced a dose-related antagonism of the anticonvulsant effects of diazepam against pentylenetetrazole (80 mg/kg) induced seizures. A dose of 30 mg/kg of βCCt (**195**) did not produce a further shift in the diazepam dose–effect curve, apparently because βCCt (**195**) failed to block the muscle relaxant effects of diazepam. Furthermore, **195** (30 mg/kg) failed to antagonize the ataxic effects of diazepam in the inverted wire screen test.

βCCt (**195**) did selectively antagonize the effects (anxiolytic) of diazepam on punished behavior as well as the anticonvulsant effects of Valium; however, **195** failed to antagonize the rate-decreasing and ataxic effects of diazepam. These results suggested that βCCt (**195**) was a selective Bz_I benzodiazepine receptor antagonist employing the earlier terminology of Beer and Lippa.[153,169]

The response–rate-decreasing effects of diazepam under both the punished and unpunished components of the multiple schedule occurred at doses which produced ataxia as measured on the rotarod in rats and are likely a direct result of the sedative, muscle relaxant, and ataxic effects of diazepam. Consequently, the ineffectiveness of βCCt **195** in blocking the rate-decreasing effects of diazepam is further evidence that **195** failed to block the ataxic and sedative effects of diazepam. This selectivity of the antagonist actions of βCCt (**195**) distinguished it from βCCE (**188**) or βCCM (**218**). The latter two β-carbolines antagonized the diazepam- or phenobarbitol-induced impairment of motor performance in the horizontal wire test in mice.

In summary, βCCt (**195**) antagonized the anticonvulsant and antipunishment (anxiolytic) effects of diazepam but not the ataxic, sedative, and muscle-relaxant effects. It was proposed in the early 1980s[153,169] that Bz_I receptors mediated the anxiolytic and anticonvulsant properties of benzodiazepines, whereas Bz_{II} (type II) receptors mediated the depressant and ataxic effects of the benzodiazepines. Within the framework of this early constraint, βCCt (**195**) appeared to be a Bz_I receptor antagonist. Although

Scheme 18



the antagonist properties of **195** are as efficacious as those of the clinically employed flumazenil (Ro 15-1788) βCCt (**195**) appeared to be more Bz_I selective than the Roche antagonist.

The long half-life *in vivo* of βCCt (**195**) when compared to βCCE (**188**) rendered **195** an important pharmacological probe of sleep[170] and other processes mediated by the CNS.[171] Because physostigmine had been reported to reverse the sedation and paradoxical delirium induced by the 1,4-benzodiazepines, Hoffman employed βCCt (**195**) to study this phenomenen. Hoffman[171] found that physostigmine increased cerebral blood flow (CBF) and cerebral oxygen consumption (CMR_{O_2}) in the CNS as reported previously. The increase in CBF was linked primarily to increases in CMR_{O_2} and a normal coupling between blood flow and metabolism. The benzodiazepine agonist midazolam, decreased CBF and CMR_{O_2} in a dose-related manner, and this decrease was antagonized by physostigmine. βCCt (**195**) increased CBF and CMR_{O_2} but this change was further potentiated by physostigmine.[171] The additive effects of βCCt (**195**) and physostigmine suggested that physostigmine antagonized the central action of benzodiazepines by a central stimulatory action, and not by a direct competitive effect at the BzR as previously suggested by others. If physostigmine produced stimulation by an action at the central $BzR/GABA_A$ complex, this action was probably independent of the BzR and in keeping with the cholinergic properties of physostigmine.

D. Pharmacophore Models of the Benzodiazepine Receptor Site

The practice of incorporating biofunctionality into a rigid framework to enhance activity or selectivity of action is often employed when trying to define a pharmacophore for a specific receptor site. The early work of Bentley et al.[172,173] in the morphine area is an excellent example of this. Previous structure–activity relationship (SAR) studies[174–177] suggested that one necessary criteria for high-affinity binding of ligands to benzodiazepine receptors was the ability of these molecules to assume a planar or pseudoplanar topography.[174,175] With these goals in mind Trudell et al.[178] in 1987 first reported the biological activity of the rigid planar 7,12-dihydropyrido[3,2-*b*:5,4-*b*']diindole (**202**) the synthesis of which is illustrated in Scheme 18.

The tetrahydro-β-carboline **196** required for this synthesis was prepared in 84% yield from tryptamine **3** via a Pictet–Spengler reaction with glyoxylic acid monohydrate (Scheme 18). Protection of the amine function at N-2 by treatment of the tetrahydro-β-carboline **196** with benzoyl chloride furnished the two rotomeric benzoyl-1,2,3,4-tetrahydro-β-carbolines represented by **197** in 79% yield. The benzamide **197** which resulted was then oxidized[179] regioselectively at C-4 with dichlorodicyanoquinone (DDQ) in aqueous THF at −78 °C to furnish the 2-benzoyl-4-oxo-1,2,3,4-tetrahydro-β-carboline **198**, in 56% yield.[99] The execution of this procedure at low temperature provided the regioselectivity of this process; however, it required reaction with DDQ as a slurry. Treatment of the 4-oxo ketobenzamide with phenylhydrazine resulted in the formation of hydrazone **199** in excellent yield. When the hydrazone **199** was heated, the obligatory [3,3] sigmatropic rearrangement of the Fischer indole reaction took place to provide the indole analog **201**. Removal of the amide functionality was accomplished by heating indolobenzamide **201** in the presence of hydrazine to furnish the fully aromatic dihydropyridodiindole (**202**) in excellent yield.

The dihydropyridodiindole (**202**) was found to bind tightly to the BzR (4 nM) and exhibited inverse agonist activity reminiscent of the activity of βCCE (**188**) and FG 7142. The rigid nature of this ligand permitted the effective superposition (using a template approach) of 12 other inverse agonists via the active analog approach of Marshall,[180,181] to arrive at the topography of the inverse agonist/antagonist site in the binding cleft at the BzR (Figure 6). An included volume analysis of these ligands resulted in the inverse agonist/antagonist pharmacophore illustrated in Figure 7. In addition to the pyridodiindoles, >100 3-substituted β-carbolines have been synthesized and modeled[40–42,44] in our laboratory to define the topography of the inverse agonist/antagonist BzR pharmacophore receptor model illustrated in Figure 7.

The results of this study suggested for potent inverse agonist activity the involvement of an indole