The Pictet-Spengler Reaction and Biogenic Tryptamines: Formation of Tetrahydro-β-carbolines at Physiological *p*H

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Biogenic tryptamines **1a-c** were reacted with aldehydes **2a & b** and α-keto acids **2c & d** to form 1,2,3,4-tetrahydro-β-carbolines (THBCs) **4d-i**, and other products, in a buffered solution at 37° and *p*H 7.4. These reactions were followed over time by ^{1}H nmr through integral changes in discrete signals in the spectra. Reactions between tryptamines and acetaldehyde (**2b**) gave the expected 1-methyl-THBCs **4d-f**, while those with sodium glyoxylate (**2c**) resulted in THBC-1-carboxylic acids **4g-i**. Surprisingly, reactions with sodium pyruvate (**2d**) or formaldehyde (**2a**) did not form the expected products **4a-c** or **4j-l**, respectively under these conditions. In successful reactions, 5-methoxytryptamine (**1c**) was found to be more reactive than tryptamine (**1a**) or serotonin (**1b**). MOPAC calculations were employed to investigate reaction intermediates. These results are applicable in research related to aberrant tryptamine metabolism; *e.g.* depression and alcoholism.

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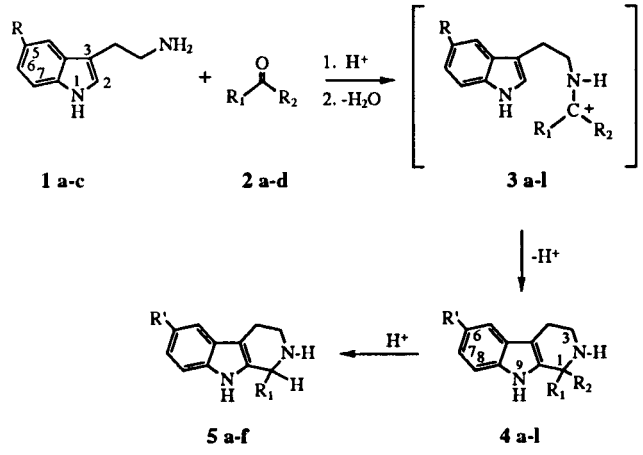
β-Carbolines (9*H*-pyrido[3,4-*b*]indoles) are considered to be natural components of many plants and animals [1] and display a broad range of biological activity [2]. Recent evidence continues to support the idea that 1,2,3,4-tetrahydro-β-carbolines (later on, THBCs) are routinely formed in humans [3], which encourages speculation on their putative role in the central nervous system (CNS). Some of the THBCs derived from tryptamine (**1a**) and 5-methoxytryptamine (**1c**) inhibit monoamine oxidase-A [4], and bind with n*M* affinity in the CNS to the serotonin transporter [5,6,7]. Thus, through entirely different mechanisms, THBCs are capable of modulating serotonergic activity by preventing the metabolism of serotonin (**1b**) and its neuronal reuptake, respectively. This is important since abnormalities in serotonergic activity have been implicated in a host of neurologic disorders, including psychiatric illnesses such as depression [8,9], neurodegenerative diseases like Alzheimer's and Parkinson's diseases [10], and alcoholism [11]. Also, β-carbolines have been implicated, at least to some extent, in all of these disorders [12,13,14]. As hypothesized earlier, this may be especially relevant in the case of alcoholism, where abundant amounts of acetaldehyde (**2b**) are available to react with tryptamines **1a-c** to form the 1-methyl-THBCs **4d-f** (later on, 1-Me-THBCs) [15,16].

The tryptamines **1a-c** are readily found in humans. Small amounts are taken in directly through the diet, while most are derived from the essential dietary amino acid tryptophan. Conventional wisdom suggests that endogenous THBCs are formed nonenzymatically through a Pictet-Spengler condensation between these tryptamines and carbonyl substrates [17] (see Scheme), similar to the formation of tetrahydroisoquinolines from phenethylamines [18]. This reaction is especially favorable towards phenethylamines under acidic conditions, and speculations on the endogenous formation of THBCs from tryptamines are routinely extrapolated from these results, though reports of careful investigations into similar reactions with tryptamines at physiological *p*H (7.4) are lacking. In our continuing efforts to investigate the ocurrence and actions of endogenous THBCs, we decided to examine more closely the reaction deemed responsible for their biological ocurrence; *i.e.* the Pictet-Spengler reaction. Reported are the results of individual reactions between tryptamines **1a-c** with two common aldehydes (formaldehyde **2a** and acetaldehyde **2b**) and two sodium salts of α-keto acids (glyoxylate **2c** and pyruvate **2d**) as likely biological carbonyl sources. Also reported, and discussed, are the results of MOPAC [19] point charge calculations of the carbon atoms connected prior to cyclization.

Results and Discussion

Adduct formation is presented graphically for the formation of the THBC-1-carboxylic acids **4g-i** (later on, 1-COOH-THBCs) from **2c** and **1a-c** (Figure 1), and the formation of 1-Me-THBCs **4d-f** from **2b** and **1a-c** (Figure 2), over time. Overall reactions with **2c** as the substrate were initiated faster (as determined by the y-intercept) and proceeded at a faster rate, than reactions with **2b**, and in both cases **1c** was found to be more reactive than either **1a** or **1b** (Table 1). Tryptophan was not sufficiently soluble under these conditions and excluded on this basis.

Scheme



1 a-c 2 a-d 3 a-l

5 a-f 4 a-l

Compound	R	R_1	R_2	Common names
1a	H	-	-	tryptamine
1b	OH	-	-	serotonin, 5-HT
1c	OMe	-	-	5-MeO-tryptamine
2a	-	H	H	formaldehyde
2b	-	Me	H	acetaldehyde
2c	-	H	COO-	glyoxylate (Na)
2d	-	Me	COO-	pyruvate (Na)
3,4,5a	H	H	H	THBC
3,4,5b	OH	H	H	6-HO-THBC
3,4,5c	OMe	H	H	6-MeO-THBC
3,4,5d	H	Me	H	1-Me-THBC
3,4,5e	OH	Me	H	1-Me-6-HO-THBC
3,4,5f	OMe	Me	H	1-Me-6-MeO-THBC
3,4g	H	H	COOH	1-COOH-THBC
3,4h	OH	H	COOH	1-COOH-6-HO-THBC
3,4i	OMe	H	COOH	1-COOH-6-MeO-THBC
3,4j	H	Me	COOH	1-Me-1-COOH-THBC
3,4k	OH	Me	COOH	1-Me-1-COOH-6-HO-THBC
3,4l	OMe	Me	COOH	1-Me-1-COOH-6-MeO-THBC

The Pictet-Spengler reaction in the condensation of tryptamines **1a-c** with aldehydes **2a & b** or a-keto acids **2c & d** to form THBCs **4a-c**, 1-Me-THBCs **4d-f**, and 1-COOH-THBCs **4g-l** through a cationic intermediate **3a-l** which follows the formation of a Shiff base (not shown). Also shown are the THBCs obtained after acid hydrolysis of 1-COOH-THBCs, **5a-f**, derived from the α-keto acids. The common names listed do not apply to intermediates.

Favorable reactions with **2d** to form **4j-l** were not observed under these conditions. The pyruvate reactions often resulted in clear dark-red solutions of complex composition after several days at 37°.

Reactions between **2a** and **1a-c** consistently and immediately resulted in the precipitation of complex products which were not the expected THBCs **4a-c**. Instead, these products more resembled tryptamines with repeating sequences of $-CH_2-O-$ attached to the aliphatic nitrogen (Figure 3). Fairly pure samples of these products were obtained when this reaction was run for 1 minute at 0°, and none of the products had properties consistent with compounds **4a-c**. The prominent evidence for this comes

Table 1

Results of ^1H NMR Experiments of Tryptamines (**1a-c**) Reacted with Glyoxylate (**2c**) and Acetaldehyde (**2b**). All Reactions were Run in Triplicate.

		n*M* reacted (y-intercept)	n*M*/min (slope)	r2
Glyoxylate reactions				
Tryptamine	1-COOH-THBC			
1a	**4g**	2.38 nM	0.113	0.998
1b	**4h**	1.25	0.175	0.981
1c	**4i**	4.46	0.332	0.993
Acetaldehyde reactions				
Tryptaminutee	1-Me-THBC			
1a	**4d**	0.881 nM	0.067	0.994
1b	**4e**	1.014	0.081	0.960
1c	**4f**	1.851	0.149	0.982

An inclination towards formation of THBCs is indicated by the y-intercept, in nmoles. The rates were determined directly from the slopes (n*M*/minutes) of triplicate runs. Reactions with glyoxylate **2c** were well under way by the time of the first spectra and continued to proceed at a faster rate than reactions with acetaldehyde (**2b**). Of all the tryptamines, 5-methoxytryptamine (**1c**) was found to react more readily than the others for both carbonyl substrates.

from the ^1H nmr spectra, where a clear singlet for a proton on C-2 appears in the aromatic region (*e.g.*, δ 7.11, s, 1H for the product from **1a**). Ultimate characterization of these products lies beyond the scope of this paper, as it is evident that THBCs are not readily formed under these conditions by this reaction pathway.

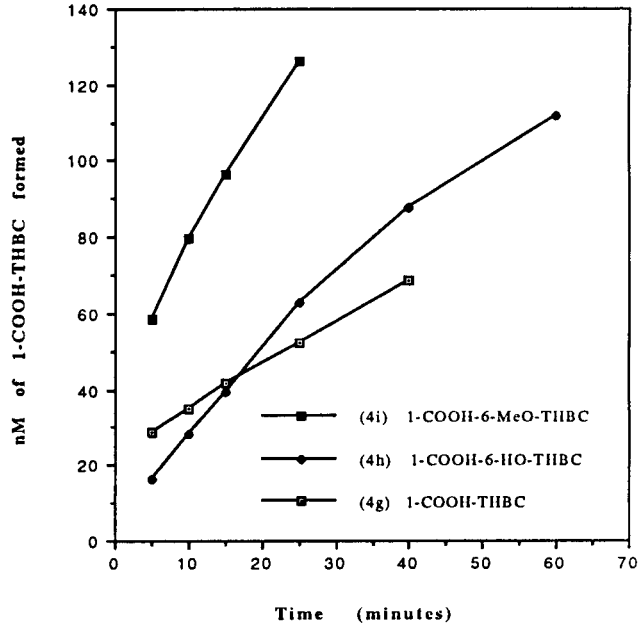


Figure 1. Formation of 1-COOH-THBCs **4g-i** over time, in nmoles, from reactions between tryptamines **1a-c** and glyoxylate (**2c**). Precipitation of the product prevented aquisition of suitable spectra from **4i** and **4g** after 25 and 40 minutes, respectively.

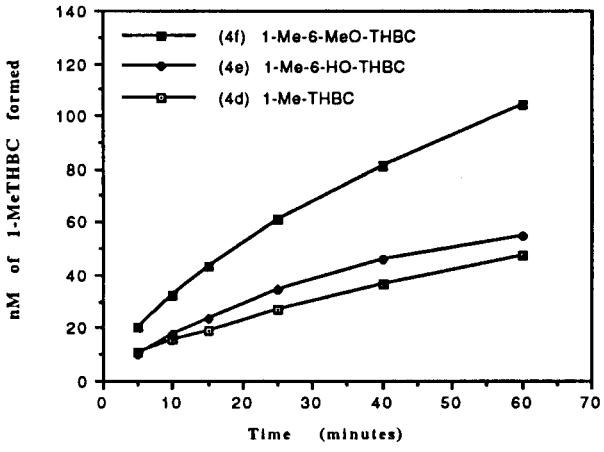
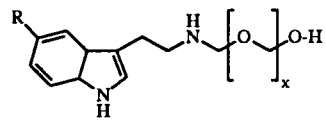


Figure 2. Formation of 1-Me-THBCs **4d-f** over time, in nmoles, from reactions between tryptamines **1a-c** and acetaldehyde (**2b**).

Half of our experimental protocol resulted in unexpected products, since we expected **1a-c** to react with both aldehydes and both α-keto acids to form THBCs, as predicted from the Pictet-Spengler reaction. To further examine this anomaly, semi-empirical calculations (AM1) were initially performed to investigate why **2b** readily reacts with tryptamines to form THBCs while **2a** does not, and ultimately to examine the electronic properties of other adducts (Table 2). The results of these calculations revealed several interesting features. For example, the indole C-2 position on the intermediate **3d** carried a negative charge (-0.064) while the carbocation had a positive charge (+0.135). Such a difference in charge is apparently sufficient for cyclization, similar results were obtained for **3e & f** (Table 2). When intermediate **3a** was calculated, the charge on C-2 was found to be -0.036 with a cationic charge of +0.063. These values for **3a** were almost half those resulting from calculations for **3d**. Similar values were obtained for intermediate **3c**, and even **3b** where the negative point charge on C-2 was quite large (-0.051), though apparently not large enough to induce cyclization.



R = H, OH, OCH_3

Figure 3. Polymeric *N*-methyloltryptamines resulted from reactions between tryptamines **1a-c** and formaldehyde (**2a**), where X increased with time and temperature.

For the sake of comparison, similar calculations were made for phenethylamine (later on, PEA), since this compound is reported to react with both aldehydes to form tetrahydroisoquinolines [17,18]. The cationic point charge for the intermediate cation of PEA with **2a** (Table 3) was not found to be much different than those for **3a-c** (Table 2). However, the crucial points were found to be at the two possible sites for cyclization on the aromatic ring of PEA, *ortho* to the ethylamine side chain, where presumably the reaction takes place at the most negative site. Here, the more negative of the two point charges for the intermediate of PEA, with **2a** as the substrate, was found to be -0.160, and with **2b** as the substrate -0.148. This is roughly four times more negative than C-2 positions on **3a-c**, while C-2 on intermediates **3d-f** was only about 2.5 times more negative. In addition, the absolute differential in charge between the cation and C-2 was quite large for intermediates **3d-f**, relative to **3a-c**.

With **2c** as the substrate, the more negative of the two ortho positions on PEA was -0.123, which was almost twice as negative as the charges on C-2 for **3g-i**. The effect of **2c** on the positive character of the cationic charge helps explain its reactivity towards the aryl carbon since the global differentials, that is the overall difference in charge between the cation and C-2, were all negative for **3g-i** and intermediates of PEA. That our calculations with PEA always resulted in a global negative difference between the point charges on the ortho positions and the cation, and that the absolute difference was relatively

Table 2

MOPAC Calculations of Point Charges (cation and C-2) on Tryptamines **1a-c** and Intermediates (int.) **3a-l**, and the absolute differentials (abs. diff.) between these values.

	Intermediates of **1a**				Intermediates of **1b**				Intermediates of **1c**		
	charges	abs.			charges	abs.			charges	abs.	
int.	cation	C-2	diff.	int.	cation	C-2	diff.	int.	cation	C-2	diff.
3a	0.063	-0.036	0.099	**3b**	0.073	-0.051	0.124	**3c**	0.062	-0.030	0.092
3d	0.135	-0.064	0.199	**3e**	0.135	-0.056	0.191	**3f**	0.136	-0.056	0.192
3g	0.011	-0.074	0.085	**3h**	0.013	-0.065	0.078	**3i**	0.013	-0.066	0.079
3j	0.054	-0.071	0.125	**3k**	0.048	-0.049	0.097	**3l**	0.054	-0.062	0.116
1a	-	-0.081	-	**1b**	-	-0.073	-	**1c**	-	-0.072	-

Values obtained from point charge calculations (MOPAC 5.0) of the carbon atoms connected just to cyclization; the C-2 carbon on the indole ring, and the intermediate carbocation attached to the aliphatic nitrogen of the intermediate **3a-l**, and the point charges for C-2 on the unreacted 1° tryptamines **1a-c**. Also shown are the absolute differences in charge between the cation and C-2.

Table 3

MOPAC Calculations of Point Charges (cationic and aryl positions) for PEA and its intermediates. Differentials were Calculated from the More Negative Aryl Value

substrate	cationic charge	charge on *ortho* positions	absolute differential	global differential
2a	0.062	-0.160, -0.112	0.222	-0.098
2b	0.124	-0.148, -0.114	0.272	-0.024
2c	0.006	-0.123, -0.123	0.129	-0.117
2d	0.047	-0.124, -0.122	0.171	-0.077
1° amine	-	0.138, -0.126	-	-

Values obtained from point charge calculations (MOPAC 5.0) of the carbon atoms connected prior to cyclization for phenethylamine (PEA); the two *ortho* positions on the aromatic ring, and the intermediate carbocation on the aliphatic nitrogen, and the point charges on the atoms *ortho* to the ethylamine side chain of unreacted PEA. The absolute and global differentials were calculated from the point charge value of the most negative *ortho* carbon.

large, is consistent with earlier observations showing that this reaction clearly favors the cyclization of PEAs over tryptamines [20 and references therein].

The unreactivity seen with **2d** presents a special case, and our observations are in agreement with earlier work published which demonstrated that **2d** does not readily condense with **1a** between *p*H 7.1-7.4 [21]. Unlike **2c**, **2d** lacks a proton on the β carbon and must form an enol before addition of the amine to the subsequent double bond [reference 13 in 17]. And unlike reactions with **2a**, **2d** does react with tryptamines under weakly acidic conditions and readily decarboxylates to 1-Me-THBCs nonenzymatically [22].

The polymeric products (Figure 3) from reactions between **2a** and **1a-c** were unexpected. Apparently the reaction would require a much lower *p*H to form the iminium Shiff base, which is essential for cyclization [23,24], and unlike **2b**, **2a** is exceptional in that it lacks α-hydrogens on the carbon adjacent to the carbonyl and the intermediate can not be stabilized through hyperconjugation [25]. Also, with **2a** as the substrate, the native point charge on C-2 may not be sufficiently negative for tryptamines **1a-c** to attract the intermediate cations of **3a-c** into seeking stability to form THBCs **4a-c** through electrophilic cyclization. Instead, the intermediate cations seek additional formyl carbons under these conditions.

Thus, when making the common assumption that THBCs can be made from tryptamines and aldehydes, one should bear in mind the reaction conditions, particularly *p*H. This same tendency of tryptamines to react with **2a** was a major obstacle to the initial verification and acceptance of THBC production *in vivo*, since trace amounts of **2a** can be generated by biological samples during the work up and typically persists in many extraction solvents, also these extraction procedures were inevitably under very acidic conditions [26].

The results from reactions with **2b** are especially intriguing in the case of excessive ethanol consumption, since approximately 0.9 g of **2b** can result from every gram of ethanol consumed. It has been shown that acute and chronic increases in **2b** result in the endogenous formation of 1-Me-THBCs in humans and other mammals [14,20,21,27-30], and an acetaldehyde-induced imbalance between 1-Me-THBCs and 1-H-THBCs seems reasonable. This hypothesis is also encouraged by the reactivity seen with acetaldehyde (**2b**), relative to glyoxylate **2c** in regard to these tryptamines **1a-c**, and by the fact that the pharmacology of 1-Me-THBCs and 1-H-THBCs differ markedly [3-14].

EXPERIMENTAL

Analyses of the reactions were made by ^{1}H and ^{13}C nmr, spectra were recorded over time on a Bruker AM 400/Aspect 3000 nmr spectrometer operating at 400.134 MHz for ^{1}H and 100.614 MHz for ^{13}C spectra. The spectra were acquired using 32 kW data points with zero filling to point resolution better than 0.2 Hz. Identities of all final products were further confirmed by EI mass spectrometry, recorded on a Trio-2-VG Masslab spectrometer (Manchester U.K.) by direct injection probe at 70 eV with an ion source temperature of 200° and an electron current of 200 μA. Elemental compositions of the ions were confirmed by accurate mass measurements and were in agreement with previous work [31].

For the time course reactions, equimolar concentrations of tryptamine (tryptamine hydrochloride and 5-methoxytryptamine hydrochloride, Sigma; or serotonin creatinine sulphate, Merck) and carbonyl substrate (formaldehyde 37% aqueous with 10% methanol, acetaldehyde 99%, Merck; sodium glyoxylate, Fluka; or sodium pyruvate, Boehringer), were reacted in a 5 mm nmr tube; 0.64 mmoles of the dissolved tryptamine (as a 400 μl aliquot taken from a 1.6 *M* aqueous solution, *p*H 7.4) was added to 700 μl of buffer (0.1 *M* aqueous sodium phospate, *p*H 7.4), followed by 100 μl of TSP (sodium-3-(trimethylsilyl)-2,2,3,3[d_4]-propionate, Merck) and 100 μl deuterium oxide (Merck). Finally, 0.64 mmoles of the carbonyl substrate was added as a 50 μl aliquot taken from a 12.8 *M* aqueous solution. The nmr tube was briefly shaken (t = 0 minutes) to mix the reactants prior to placement into the probe (at 37°). After shimming the instrument, the initial ^{1}H spectra were obtainable after 3 minutes. Data from the integrated spectra of these reactions were plotted as a normalized percentage of the product formed, expressed in nmoles, versus time, in minutes. The nmolar amount of THBC formed, and tryptamine reacted, were calculated directly from the integral value of single proton signals in the aromatic region of the spectra. Precipitation of the product typically marked the end of a successful reaction, as well as the ability to obtain good spectra. All reactions were run in triplicate. Acetaldehyde was distilled prior to use.

Additional ^{1}H nmr analyses were made of products resulting from reactions with the α-keto acids **2c & d**, following decarboxylation of the resulting 1-COOH-THBCs **4g-l** to their corresponding 1-H-THBCs **5a-f** by the addition of 100 μl 37%

hydrochloric acid.

For analytical purposes 0.100 g (0.51 mmole) of **1a** hydrochloric acid was reacted with an equimolar amount of **2a** for one minute at 0°, since higher temperatures and longer reaction times resulted in complex resins. A fine-white electrostatic powder was recovered (0.107 g) after filtration and desiccation, which foamed and decomposed over a wide temperature range (85-125°). This foam was a red oil by 165° and a clear dark-red oil by 290°. The product did not dissolve in common organic solvents, and decomposed after the addition of organic and mineral acids. It was insoluble in 5 *M* sodium hydroxide and concentrated ammonium hydroxide. ^{1}H nmr (DMSO d-$_6$): δ 2.81 (br, 1H, N-H), 2.84-2.87 (m, ethyl, 4H), 3.41 (s, methylenes, 6H), 3.51 (br, 1H, OH), 6.96 (dd, 1H, 5-H, J = 7.0, 7.8 Hz), 7.05 (dd, 1H, 6-H, J = 7.1, 8.0 Hz), 7.11 (s, 1H, 2-H), 7.32 (d, 1H, 7-H, J = 8.1 Hz), 7.52 (d, 1H, 4-H, J = 7.1 Hz), 10.71 (s, 1H, ArN-H); ^{13}C nmr (DMSO d-$_6$): δ 136.2, 127.2, 122.4, 120.7, 118.3, 118.1, 112.5, 111.3, 73.7, 52.9, 51.8, 23.3; ms: m/z (relative intensities) 186.09 (9), 172.14 (23), 143.73 (base), 129.95 (25), 115.22 (6). Small amounts of impurities were evident from the nmr spectra and all attempts to purify this product resulted in more complex mixtures.

Except for substitutions on the aromatic ring, the spectral and physical properties of analogous products resulting from **1b** and **1c** were essentially identical and otherwise unremarkable (data not shown).

Structures of the intermediate reaction products **3a-l** were fully optimized using a semi-empirical MOPAC 5.0 program with AM1 parameterization [19]. Point charges were obtained from standard output and compared with calculations from analogous intermediates of phenethylamine (PEA).

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