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GAS CHROMATOGRAPHIC/MASS SPECTROMETRIC EVIDENCE FOR THE IDENTIFICATION OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE AS AN IN VIVO CONSTITUENT OF RAT BRAIN

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SUMMARY

Based on gas chromatographic/mass spectrometric data, obtained using the method of selected ion monitoring, the compound 1,2,3,4-tetrahydro-β-carboline has been tentatively identified as an in vivo constituent of rat brain.

The formation of 1,2,3,4-tetrahydro-β-carbolines (aka., tryptolines) under biomimetic conditions via the condensation of tryptamines with formaldehyde (HCHO) was reported as early as 1934 (1). The in vitro formation of 2-methyl-1,2,3,4-tetrahydro-β-carboline (2-MTHBC, V, Fig. 1) and 1,2,3,4-tetrahydro-β-carboline (THBC, VI, Fig. 1) from N-methyltryptamine (NMT, I, Fig. 1) and tryptamine (TA, II, Fig. 1), respectively, has now been repeatedly demonstrated in incubations of various mammalian tissues containing the added methyl donors 5-methyltetrahydrofolate (5-MTHF) and S-adenosylmethionine (SAM) (2-13). Investigators have concluded that the formation of the tetrahydro-β-carbolines in such studies is, however, an artifact produced by the enzymatic oxidation of the methyl donor to HCHO (2,3,6,10-15). The HCHO then presumably condenses non-enzymatically with the indole substrates via a Pictet-Spengler reaction (16) (Fig. 1) to form the β-carbolines. The formation of THBC in vivo from endogenous TA (17-21) and metabolically formed HCHO via either enzymatic or non-enzymatic mechanisms has thus remained a point in question.

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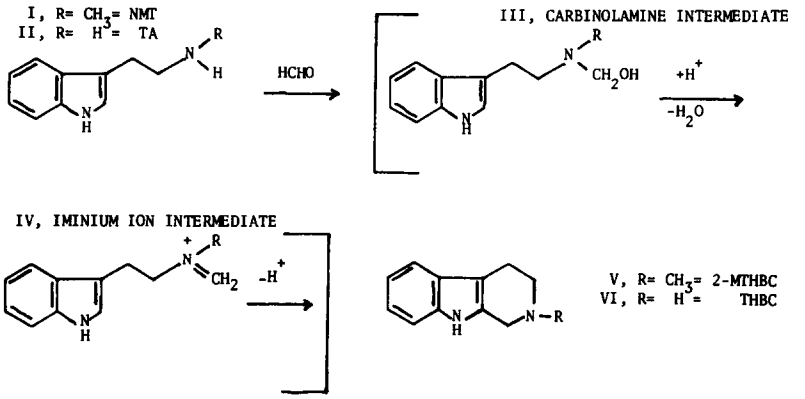


Fig. 1. Mechanism for the Pictet-Spengler reaction in the formation of tetrahydro-β-carbolines from tryptamines and formaldehyde.

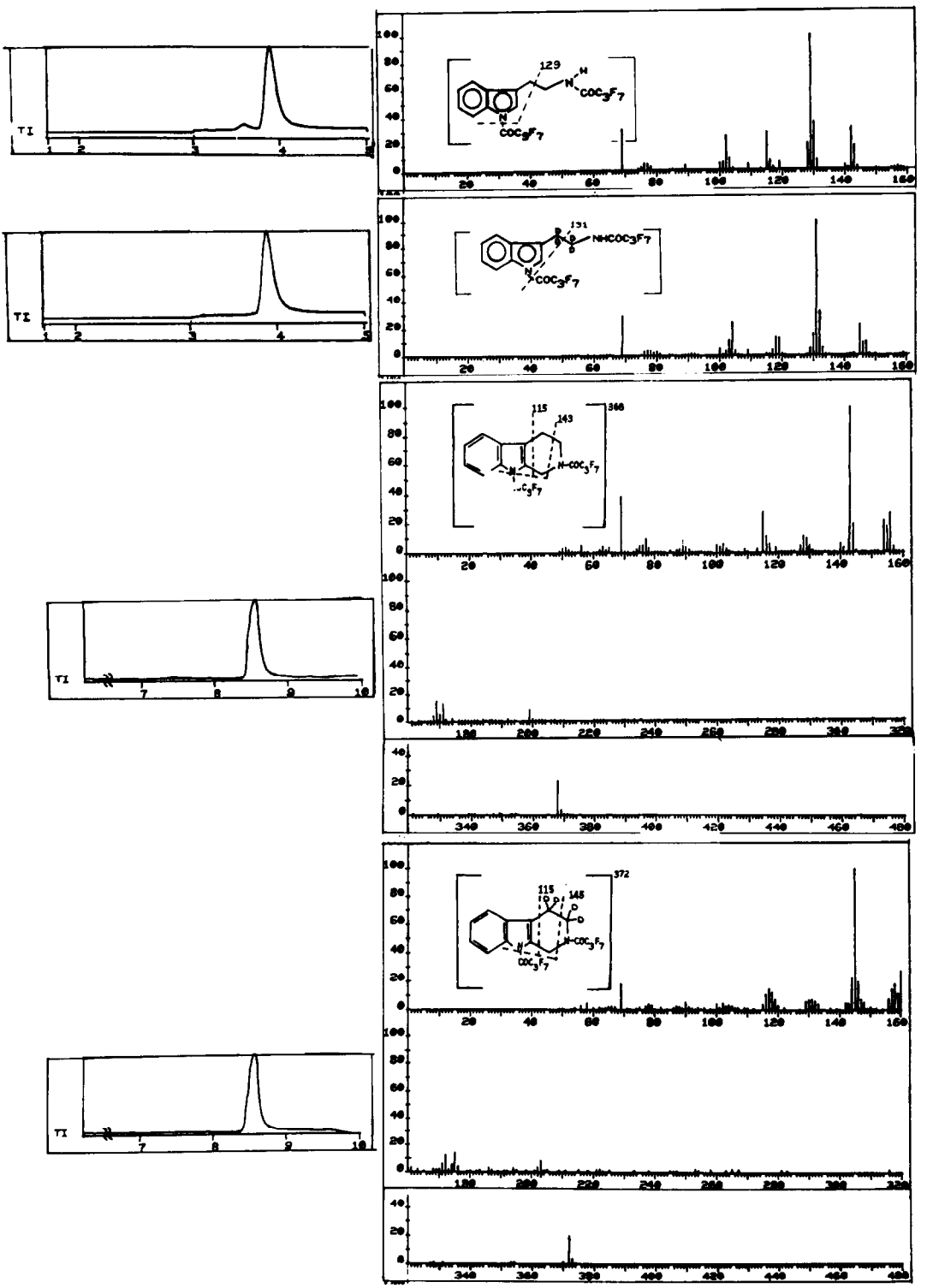
In this report we present gas chromatographic/mass spectrometric (GC/MS) evidence for the identification of 1,2,3,4-tetrahydro-β-carboline (THBC, VI, Fig. 1) as an in vivo constituent of rat brain.

MATERIALS AND METHODS

Tryptamine (TA)·HCl and glyoxylic acid were purchased from Aldrich Chemical Co., Milwaukee, Wisc. Tryptamine-α,α-d_2,β,β-d_2 (DTA)·HCl (98 atom % D_2) was purchased from Merck Sharpe and Dohme Isotopes, Montreal, Canada. Reference standards for 1,2,3,4-tetrahydro-β-carboline (THBC) and 1,2-dihydro-3,3,4,4-tetradeutero-β-carboline (TDBC) were prepared by the method of Ho and Walker and purified by repeated recrystallizations from ethanol: M.P. -204-205°C (21). Heptafluorobutyrylimidazole (HFBI) was a gift from Pierce Chemical Co., Rockford, Ill.

GC/MS of Standards: Authentic samples of TA, DTA, THBC and TDBC were quantitatively converted to their corresponding heptafluorobutyryl (HFB) derivatives (22) for GC/MS analysis. The GC/MS characteristics of these compounds were determined using a Hewlett Packard 5985 GC/MS equipped with a data analysis system. The GC was conducted on a Supelco 4', 2 mm internal diameter, glass column containing 2% SP-2250 on 100-120 mesh Chromosorb-W-HP. A stepped temperature program was used to obtain efficient separation: 150°C initial T, isothermal for 2 minutes, rising 10°C/minute to 190°C whereupon the rate of T rise increased to 30°C/minute to 250°C. High purity helium was used as the carrier gas and a flow rate of 40 ml/minute was maintained throughout the run. Electron impact (EI) mass spectra of the compounds studied were recorded by total ion (TI) monitoring of the effluent (Fig. 2) and were characterized with respect to their base peaks (normalized to 100%) and other prominent secondary mass fragments.

The retention times were recorded and the chosen mass fragments were monitored in the selected ion monitoring (SIM) mode to determine ion ratios. The mass spectrometer was tuned daily, using the m/e peaks 69.0, 219.0 and 502.0 amu of the calibration standard perfluorotributyl amine for the EI-TI studies. For the SIM studies the instrument was detuned for the 69.0 peak


TI
1 2 3 4 5
129
131
115
143
366
372
145
20 40 60 80 100 120 140 160
340 360 380 400 420 440 460 480

to maximize the sensitivity at the lower mass ranges. Reference standards were examined in the SIM mode, recording retention times and ion ratios, prior to and following injection of the rodent brain extract samples.

Preparation of Rodent Whole Brain Extract: Individual male Sprague-Dawley rats were killed by decapitation and the whole brain was rapidly (∿ 30 seconds) excised. The brain was immediately placed in a tared glass homogenizing tube which contained 1000 ng of DTA in 2.5 ml of 14% perchloric acid at 4°C. The brain was weighed and then thoroughly homogenized with a teflon pestle. The volume of the sample was brought to 5.0 ml by the addition of glass distilled water and then vigorously agitated to insure good mixing. The sample was centrifuged at 1000 x g for 20 minutes at 4°C (Sorvall RC2-B). The supernatant was saved and the pellet was further washed with 3.0 ml of 7% perchloric acid. This was recentrifuged and the supernatants were combined. The final volume was brought to 8.0 ml by the addition of glass distilled water. Salt (0.25 g NaCl) was added and the samples were vigorously shaken for 15 minutes with 2.0 ml of 15% isooctane in hexane to remove lipids. The organic layer was removed with a pasteur pipette and the aqueous phase was saturated with NaCl. The samples were placed in an ice bath and the pH was adjusted to 12 by the slow addition of 45% KOH. The resulting precipitate was removed by centrifugation (International Clinical Centrifuge) and the supernatant was extracted twice with 6 ml each of methylene chloride (CH_2Cl_2). The CH_2Cl_2 extract was dried with 3.0 g of Na_2SO_4 and evaporated in three portions in a conical flask (15 ml). The resulting residue was derivatized by the addition of 20 µl of HFBI and heating for 1 hour at 85°C (22). After cooling to room temperature the reaction was taken up in 1.0 ml of CH_2Cl_2 and washed four times with water (1.0 ml/wash). The CH_2Cl_2 layer was dried with Na_2SO_4 and a 1.5 µl aliquot of the sample was used for GC/MS analysis. The overall extraction efficiency for TA was > 90% while that for THBC was > 80% as determined by GC/MS analyses of control samples containing known amounts of each compound.

RESULTS AND DISCUSSION

The masses 129.1 and 131.1 amu were monitored for endogenous tryptamine (TA) and added deutero-tryptamine (DTA), respectively (Fig. 2, 3). The DTA was in excess of endogenous TA (see Fig. 3 for a representative graph) and thus served as an internal control for the possible artifactual formation of THBC from *in vitro* condensation of TA with HCHO. If such a reaction were to occur TDBC formation would also be observed. This possibility precluded the addition of TDBC to the samples as an internal standard.

Thus, three ions were monitored for the identification of THBC as an *in vivo* constituent of rat brain; 143.1, the base peak, 115.1, a secondary ion, and 368.2 amu, the molecular ion of the HFB-THBC derivative (Fig. 2). A fourth

Fig. 2. Gas chromatographic/mass spectrometric data for the heptafluorobutyryl derivatives of the tryptamine (TA), tetradeutero-tryptamine (DTA), 1,2,3,4-tetrahydro-β-carboline (THBC) and 1,2, dihydro-3,3,4,4-tetradeutero-β-carboline (TDBC) standards, respectively.

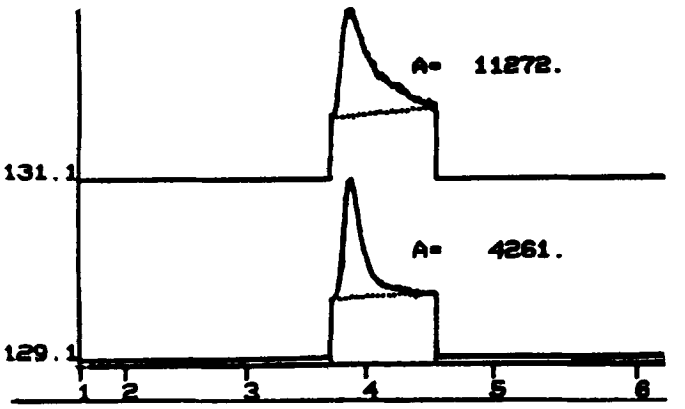


Fig. 3. Representative graph of GC/MS analyses for added DTA and endogenous TA in rat whole brain extract.

ion was monitored at 145.1 amu for the base peak of HFB-TDBC, there being < 1.0% contribution to this peak by the HFB-THBC (Table 1).

The results of the GC/MS analyses of six rodent brain extracts are presented in Table 1 and are compared to the retention time and ion ratios obtained for the THBC reference standard. A representative GC/MS graph is shown in Figure 4. Following these analyses, a spike (100 pg/μl) of HFB-TDBC was added to the samples. The mass fragments 145.1, 160.1 and 372.1 amu were then monitored for the added HFB-TDBC (Fig. 2) and the 143.1 ion was monitored for the endogenous THBC (Table 1 and Fig. 5). This provided a confirmation of retention times and served as a semiquantitative measure of endogenous THBC concentration.

The fact that the ion-current profiles of the three selected ions for THBC showed the same intensity ratio as those seen in the reference standard and that the retention times of the sample, the reference and the TDBC spike were in agreement provides good evidence for the presence of THBC in vivo (23). The low concentration (avg. = 11.8 pmoles/g wet wt. tissue) of the THBC precluded obtaining a complete mass spectrum of the peak. The addition of DTA during the homogenization step did not lead to the formation of TDBC indicating that the THBC peak observed in these studies did not arise from the condensation of endogenous TA and HCHO as an artifact of the procedure.

TABLE 1

Results of the GC/MS analyses for endogenous THBC in six rat brains.

SAMPLE	BRAIN WEIGHT (in g,wet)	RETENTION TIME (MIN)	ION RATIOS % 143.1	115.1	368.2	145.1	ION RATIO 145.1/143.1**	pm/g WET WT. BRAIN TISSUE †
THBC Standards	----	8.5	100	27±7*	5±2*	0	----	---
1	2.17	8.5	100	33.9	3.9	0	0.59	1.1
2	2.18	8.5	100	22.6	6.5	0	6.95	12.4
3	2.23	8.5	100	25.4	4.3	0	11.10	19.3
4	2.30	8.5	100	24.5	4.4	0	9.69	16.3
5	2.10	8.5	100	26.6	4.4	0	9.03	16.7
6	2.14	8.5	100	30.9	6.0	0	2.83	5.1
								Avg. = 11.8

* Ion ratios varied within these limits and were dependent on the tuning of the instrument and the method of peak area integration.

** Ratio of the 145.1 ion for TDBC and the 143.1 ion for THBC following the addition of 100 pg/µl of TDBC to the samples.

† Values are uncorrected for extraction efficiency and are reported as approximate concentrations.

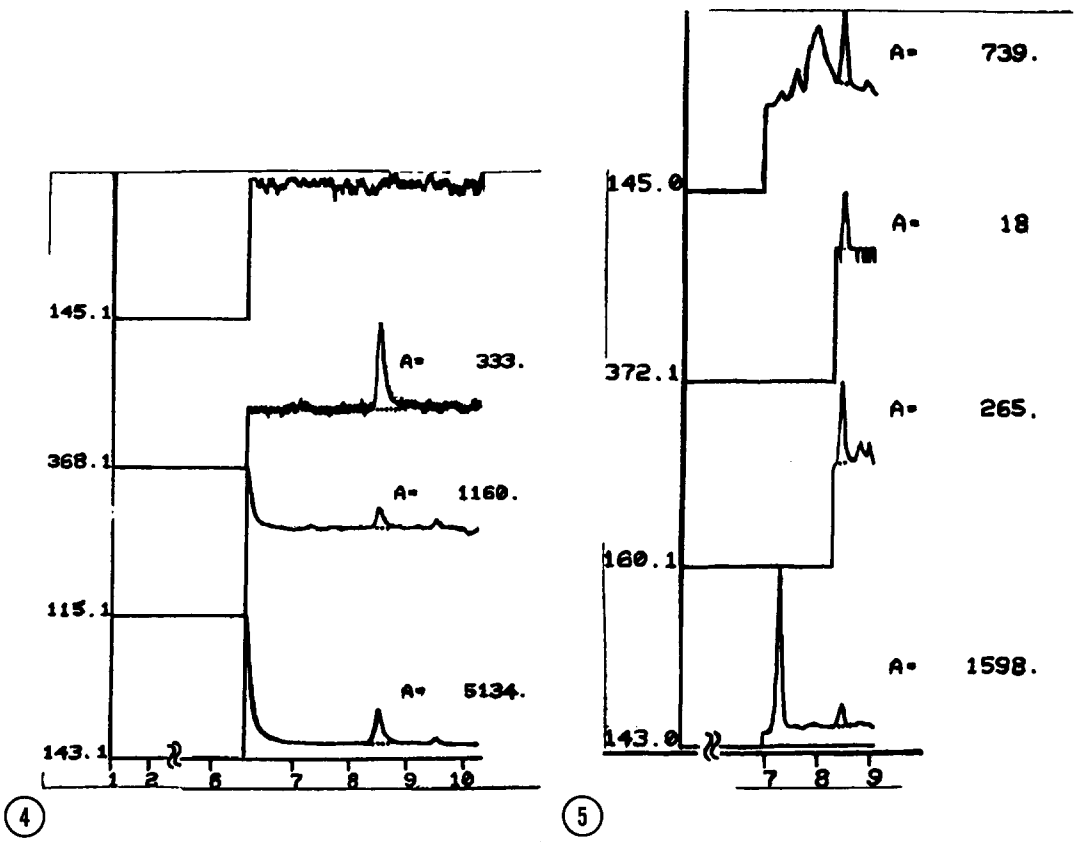


Fig. 4. Representative graph of GC/MS analyses for endogenous THBC and the possible artifactual formation of TDBC, monitored as internal control.

Fig. 5. Representative graph of GC/MS analyses of samples spiked with 100 pg/μl of TDBC-HFB as an external standard.

Although the tetrahydro-β-carbolines may be formed from simple chemical condensation in vivo, an enzymatic process involving an aldehyde and an indolamine remains a viable alternative. Furthermore, any metabolic process that would produce the carbinolamine (III, Fig. 1) and subsequently the iminium ion (IV, Fig. 1) intermediates proposed in the Pictet-Spengler reaction (16, Fig.1), possibly arising from indolalkylamine metabolism, could lead to β-carboline formation.

The formation of β-carbolines in vivo has been postulated by several investigators (2-15, 24-26). The results of this study demonstrate the in vivo presence of 1,2,3,4-tetrahydro-β-carboline (THBC) in rat brain and lend a

degree of validity to the idea that THBC may have a role in the neuroregulatory processes of mammalian brain (24, 27). For example, THBC has been reported to be both a potent MAO (28, 29) and serotonin reuptake inhibitor (30, 31). Its function in vivo may thus be to regulate MAO activity and biogenic amine levels in mammalian brain. These possibilities and others are currently under investigation.

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