Biochemical Pharmacology, Vol. 30, pp. 9–17.

0006-2952/81/0101-0009 $02.00/0

IDENTIFICATION AND QUANTIFICATION OF 1,2,3,4-TETRAHYDRO-β-CARBOLINE, 2-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE, AND 6-METHOXY-1,2,3,4-TETRAHYDRO-β-CARBOLINE AS *IN VIVO* CONSTITUENTS OF RAT BRAIN AND ADRENAL GLAND*†

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(*Received* 18 *April* 1980; *accepted* 28 *May* 1980)

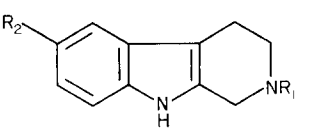
Abstract—The identification and quantification of three 1,2,3,4-tetrahydro-β-carbolines as normal constitutents of rat brain and adrenal gland, using combined gas chromatographic/mass spectrometric techniques, are reported. Qualitative analyses of these tissues led to the identification of 1,2,3,4-tetrahydro-β-carboline (THBC), 2-methyl-THBC (2-MTHBC) and 6-methoxy-THBC (6-MeOTHBC), as determined by observed peak retention times, mass fragments, and ion mass ratios. Quantitative analyses, using deuterated internal standards, gave the following results: THBC (ng/g wet wt) in brain = 17.5 ± 4.86, adrenal = 500.3 ± 163. 6-MeOTHBC (ng/g wet wt) in brain = 35.6 ± 16.6, adrenal = 1113.7 ± 300. Mechanisms for the formation of these β-carbolines as well as their possible function *in vivo* are discussed.

The formation of 1,2,3,4-tetrahydro-β-carbolines (THBCs), via the Pictet–Spengler condensation of tryptamines with formaldehyde, has been demonstrated repeatedly in incubations of various mammalian tissues containing added indolethylamine substrate and the methyl donors 5-methyltetrahydrofolate (5-MTHF) or *S*-adenosylmethionine (SAM) [1–13]. Investigators have concluded that the formation of these THBCs is an artifact produced by the enzymatic liberation of formaldehyde from the methyl donors and the subsequent non-enzymatic condensation of this formaldehyde with the indole substrates [1–3, 6, 10–15]. The formation of THBCs *in vivo* has thus remained a point of contention.

In this report we present gas chromatographic/mass spectrometric (GC/MS) evidence for the identification of THBC (I), 2-methyl-THBC (2-MTHBC, II) and 6-methoxy-THBC (6-MeOTHBC, III, Fig. 1) as *in vivo* constituents of rat brain and adrenal gland. Data concerning the quantification of these compounds in these tissues are also reported.

MATERIALS AND METHODS

Tryptamine (TA)·HCl, 5-methoxy-TA·HCl (5-MeOTA) and glyoxalic acid were obtained from the Aldrich Chemical Co., Milwaukee, WI, U.S.A. Tryptamine-α,α-d_2,β,β-d_2 (DTA) and 5-methoxytryptamine-α,α-d_2,β,β-d_2 (5-MeODTA) were obtained from Merck, Sharp & Dohme Isotopes, Montreal, Canada. An authentic sample of *N*-methyltryptamine (NMT) was provided by Professors Fred Benington and Richard Morin of this laboratory. Samples of 1,2,3,4-tetrahydro-β-carboline (THBC), 1,2-dihydro-3,3,4,4-tetradeutero-β-carboline (TDBC), 6-methoxy-THBC (6-MeOTHBC), 6-methoxy-TDBC (6-MeOTDBC) and 2-methyl-THBC (2-MTHBC) were prepared by the reaction of TA, DTA, 5-MeOTA, 5-MeODTA and NMT, respectively, with glyoxalic acid, according to the method of Ho and Walker [16]. Heptafluorobutyrylimidazole was a gift from the Pierce Chemical Co., Rockford, IL, U.S.A. All other reagents were obtained from commercial sources and were of the highest available purity.



I $R_1 = R_2 = H$ = THBC
II $R_1 = CH_3$, $R_2 = H$ = 2-MTHBC
III $R_1 = H$, $R_2 = OCH_3$ = 6-MeOTHBC

Fig. 1. Structures of the tetrahydro-β-carbolines identified in this study.

* Presented in part at the Ninth Annual Society for Neuroscience Meeting, Atlanta, GA, 1979.

† Supported by NIH Grant 5R01-HD11893.

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Gas chromatography/mass spectrometry of standards. Authentic samples of TA, DTA, 5-MeOTA, 5-MeODTA, 6-MeOTHBC, 6-MeOTDBC, THBC, TDBC, NMT and 2-MTHBC were quantitatively converted to their corresponding heptafluorobutyryl (HFB) derivatives [17] for GC/MS analysis. The GC/MS characteristics of these compounds were determined using a Hewlett Packard 5985A GC/MS equipped with a data analysis system. Gas chromatography was conducted on a Supelco 4 ft, 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: 200° initial temperature, isothermal for 3 min, whereupon the temperature was raised to 250° at a rate of 20°/min. High purity helium was used as the carrier gas, and a flow rate of 40 ml/min was maintained throughout the run.

Electron impact (EI) mass spectra of the compounds were recorded by total ion (TI) monitoring of the effluent. Each compound was characterized with respect to its base peak (normalized to 100 per cent) and other prominent secondary mass fragments. The retention times were recorded and the chosen diagnostic mass fragments were monitored for each compound in the selected ion monitoring (SIM) mode. Ion ratios were noted and the per cent cross-talk between the ions chosen for the proteo and deutero compounds was calculated. Reference standards were examined in the SIM mode, recording retention times and ion ratios, prior to and following injection of the samples obtained from the tissue extracts.

Preparation of rat brain and adrenal gland extracts. Male Sprague–Dawley rats (120 days old) were housed singly for 21 days and were fed and watered *ad lib.* The animals were decapitated, and the brain and adrenal glands were rapidly excised. The brain and adrenal glands from individual rats were immediately placed in separate, tared glass homogenizing tubes that contained 1000 ng each of DTA and 5-MeODTA in 2.5 ml of 14% perchloric acid at 4°. The tissues were weighed and then thoroughly homogenized with a Teflon pestle. The samples were treated further and extracted as described previously [18]. These samples were used for the qualitative analysis of β-carbolines in these tissues and for monitoring the possible artifactual formation of β-carbolines during the work-up procedure.

A second set of pooled tissue samples (eight brains, sixteen adrenals) was spiked with increasing concentrations of proteo standards for THBC and 6-MeOTHBC while holding the corresponding deutero standard concentration constant (1000 ng each). The samples were then homogenized and treated as described previously [18]. These samples were used to determine the linearity of recovery and correlation of ion ratios for the respective compounds.

A third set of tissue samples was spiked with 1000 ng each of 6-MeOTDBC and TDBC, homogenized and treated as described [18]. These samples were used to quantify endogenous THBC and 6-MeOTHBC by comparing ion mass ratios between the endogenous material and the added deutero standards. Due to the unavailability of deuterated NMT we were unable to quantify 2-MTHBC by this method.

RESULTS AND DISCUSSION

GC/MS of standards. The retention times and mass spectra of 2-MTHBC, THBC and TDBC, and 6-MeOTHBC and 6-MeOTDBC are shown in Figs. 2, 3 and 4 respectively. The ions *m/e* 143.1, 144.1, 186.2 and 115.1 were chosen to monitor for the presence of endogenous 2-MTHBC. The ions *m/e* 143.1, 368.1, 156.1 and 115.1 were used to monitor for THBC. The ions *m/e* 145.1 and 160.1 were chosen for TDBC, there being less than 0.1 per cent cross-talk between these ions, and the ions *m/e* 143.1 and 156.1 for THBC. The ions selected for 6-MeOTHBC were *m/e* 173.1, 398.0, 158.1 and 185.1. The ions *m/e* 175.1 and 160.1 were selected for monitoring 6-MeOTDBC.

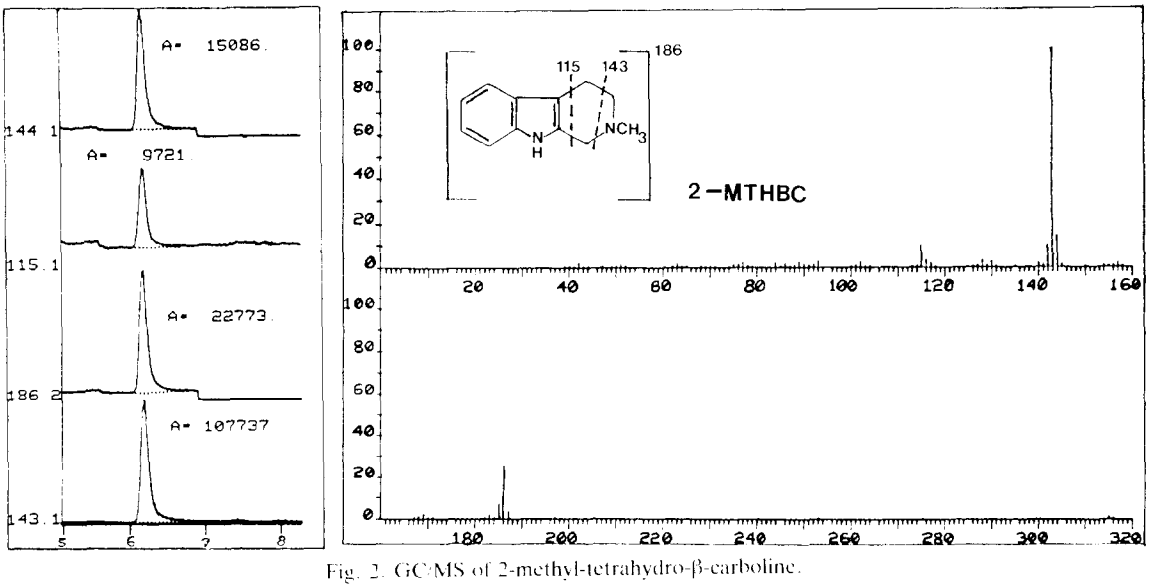

Fig. 2. GC/MS of 2-methyl-tetrahydro-β-carboline.

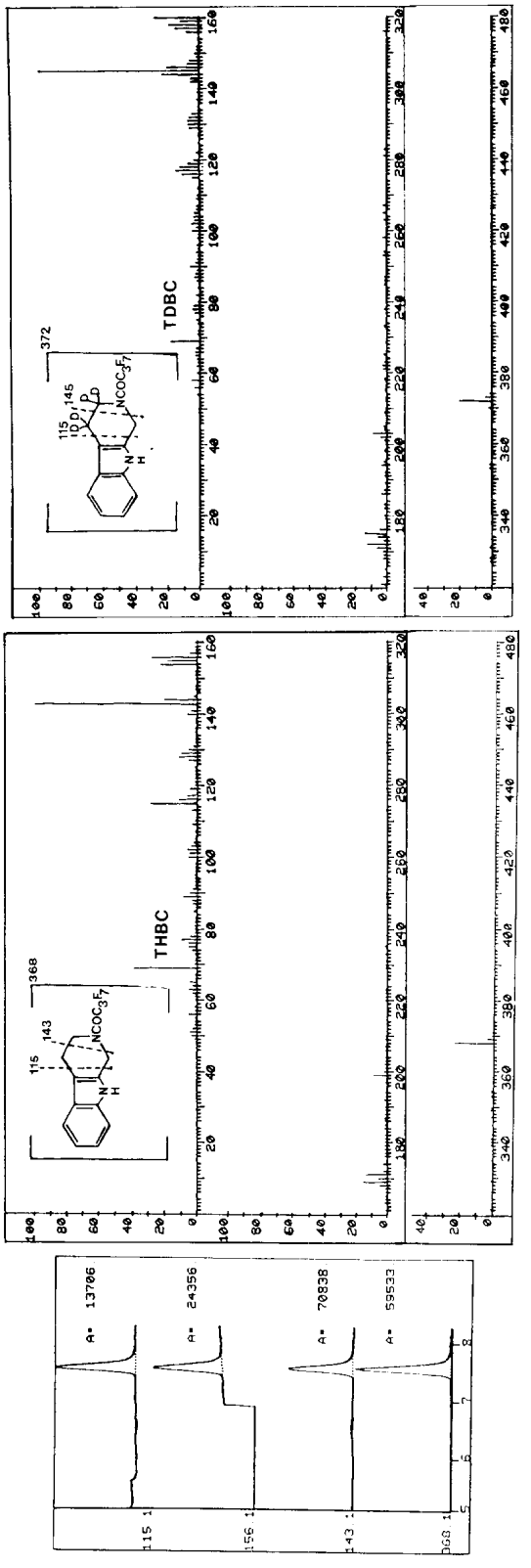

Fig. 3. GC/MS of tetrahydro-β-carboline and tetradeutero-β-carboline.

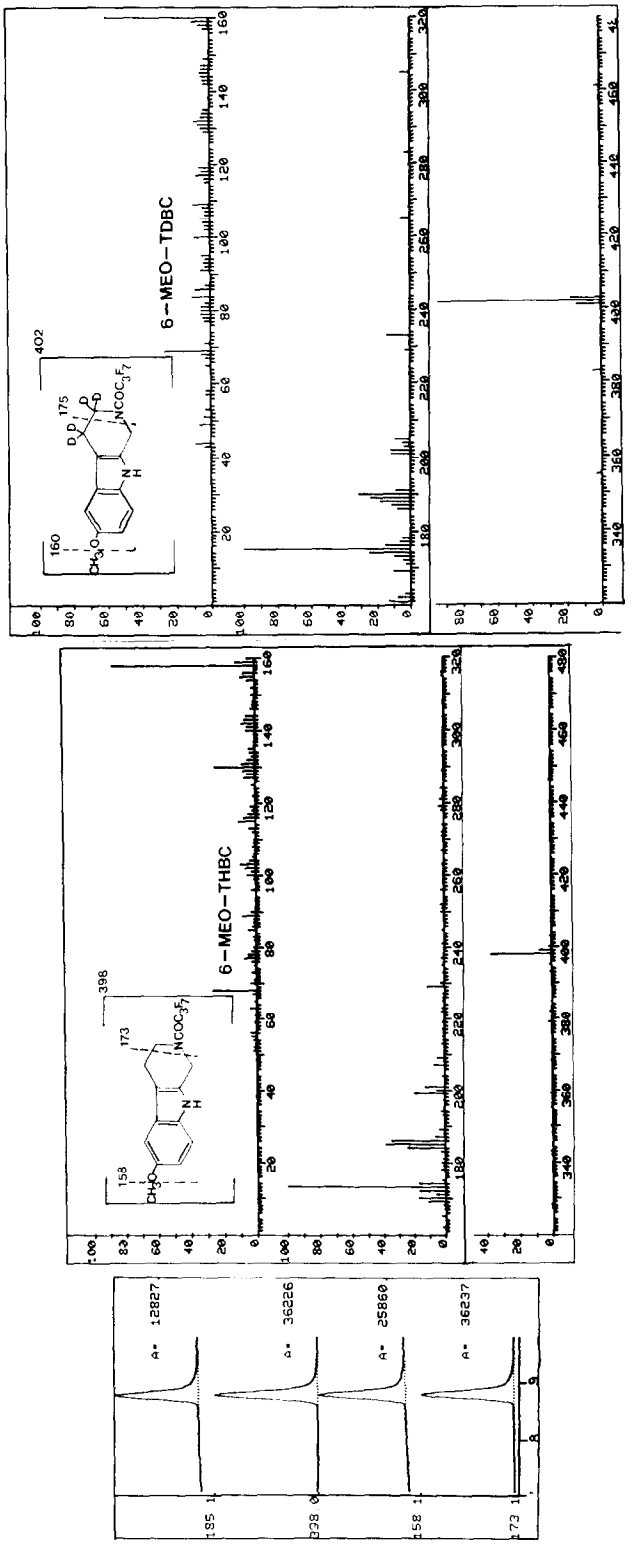

Fig. 4. GC/MS of 6-methoxy-tetrahydro-β-carboline and 6-methoxy-tetradeutero-β-carboline.

Qualitative analysis of rat brain and adrenal gland extracts. Qualitative analysis of rat brain and adrenal extracts led to the identification of 2-MTHBC, THBC, and 6-MeOTHBC as normal constituents of these tissues (Fig. 5). The peaks observed had the same retention times, mass fragments and ion mass ratios as those observed for the standards (Table 1). Added DTA and 5-MeODTA served as internal controls for the possible artifactual formation of THBC and 6-MeOTHBC from the *in vitro* condensation of endogenous TA and 5-MeOTA with endogenous formaldehyde. If such a reaction were to occur, TDBC and 6-MeOTDBC formation would also have been observed. In agreement with our preliminary results [18], the addition of DTA or 6-MeODTA did not lead to the formation of TDBC or 6-MeOTDBC, indicating that the THBC and 6-MeOTHBC peaks observed in these studies did not arise from the condensation of endogenous TA or 5-MeOTA with HCHO as an artifact of the procedure.

The recoveries of THBC and 6-MeOTHBC from brain or adrenal tissues were observed to be linear with increasing concentration (Fig. 6). The point of intercept on the ordinate of Fig. 6 represents the ratio of endogenous THBC and 6-MeOTHBC to the added deutero standards in the pooled tissue samples (see Materials and Methods).

Quantification of THBC and 6-MeOTHBC in rat brain and adrenal gland extracts. A representative chromatogram for the quantification of THBC and 6-MeOTHBC is presented in Fig. 7. The results of the analyses of individual rat brain and adrenal gland samples are presented in Table 2. The ratio of the ions *m/e* 156/160 was used to quantify THBC, while the ions *m/e* 173/175 were used to quantify 6-MeOTHBC. These ions were chosen because of the excellent correlation of ion ratios for the respective compounds (Fig. 6).

Localization of 6-MeOTHBC and THBC in rat brain. Qualitative GC/MS evidence for the identification of 2-MTHBC and THBC as normal constituents of rat brain was previously reported by this laboratory [19]. The identification of THBC in rat brain was subsequently confirmed [18, 20]. Honecker and Rommelspacher [20] have estimated the concentration of THBC in rat forebrain as being 47.3 ng/g. This value was obtained by labeling the extracted THBC with [^{3}H]acetic anhydride, followed by t.l.c. isolation and scintillation counting. Using a semi-quantitative GC/MS technique, Barker *et al.* [18] estimated the level of THBC to be 2.0 ng/g of rat whole brain. Shoemaker *et al.* [21] have presented evidence suggesting the identification of 6-MeOTHBC and 1-methyl-β-carboline as naturally occurring substances in the rat arcuate nucleus, using a He-Cd ultraviolet laser microfluorimeter and t.l.c. techniques. Honecker and Rommelspacher [20] have reported, however, that 6-MeOTHBC was apparently not a constituent of rat forebrain.

The difference in the quantity of THBC reported in this study (17.5 ng/g) for rat whole brain and that reported for THBC in rat forebrain (47.3 ng/g) [20] may indicate that THBC is more concentrated or localized in forebrain tissues. This appears to be the case for the apparent precursor for THBC, TA, with highest levels being located mainly in the caudate nucleus [22]. Similarly, with the identification of 6-MeOTHBC in the rat arcuate nucleus and its apparent absence in rat forebrain tissues, 6-MeOTHBC may be more localized in mid- and hindbrain structures. Indeed, Prozialeck *et al.* [23] have observed 5-MeOTA, the apparent precursor for 6-MeOTHBC, to be located mainly in mid- and hind-

Table 1. Qualitative analyses of rat brain and adrenal glands for 2-MTHBC, THBC, and 6-MeOTHBC

Sample		Ions (*m/e*) and ion ratios (%)				Retention time (min)
2-MTHBC		143.1	186.2/143.1	115.1/143.1	144.1/143.1	
Standards		100	21 ± 8	9 ± 9	14 ± 4	6.2
Adrenal	1	100	18.8	19.1	16.5	6.2
	2	100	30.8	20.9	16.0	6.2
Brain	1	100	25.3	18.3	15.4	6.2
	2	100	20.7	14.1	14.4	6.2
THBC		143.1	368.1/143.1	156.1/143.1	115.1/143.1	
Standards		100	84 ± 5	41 ± 5	19 ± 3	7.6
Adrenal	1	100	83.1	30.8	18.6	7.6
	2	100	90.1	43.1	17.6	7.6
Brain	1	100	86.3	39.2	19.1	7.6
	2	100	89.1	41.0	18.5	7.6
6-MeOTHBC		173.1	158.1/173.1	398.0/173.1	185.1/173.1	
Standards		100	71 ± 4	100 ± 6	35 ± 3	8.8
Adrenal	1	100	69.0	106.0	31.2	8.8
	2	100	67.9	101.1	33.5	8.8
Brain	1	100	69.8	100.0	32.1	8.8
	2	100	71.0	98.8	34.7	8.8

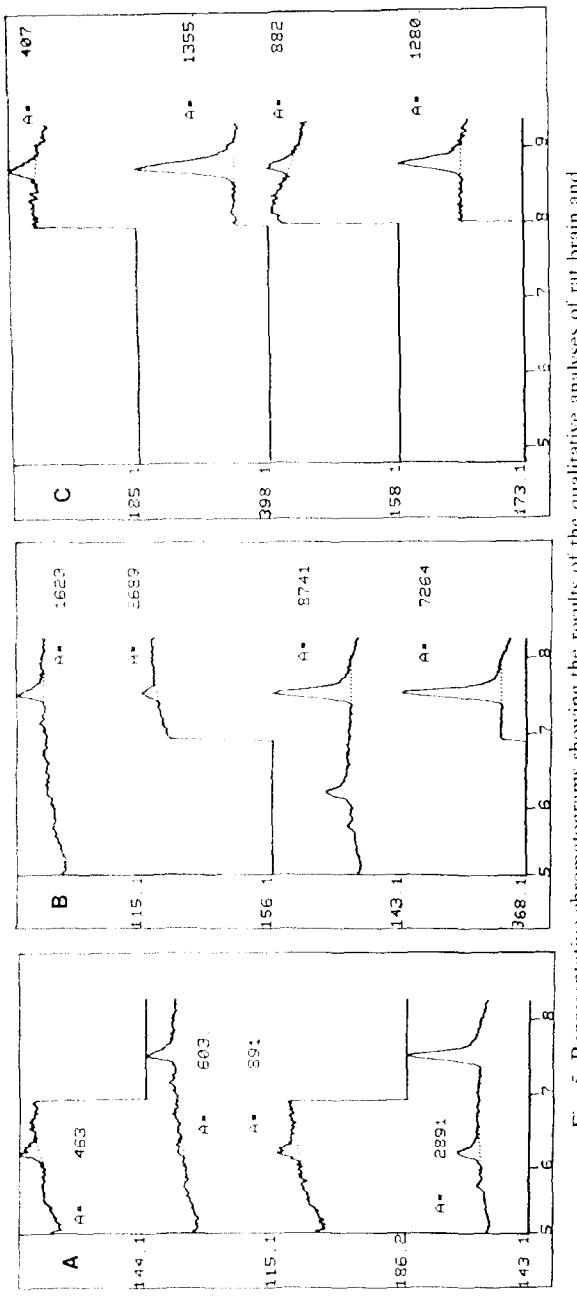

Fig. 5. Representative chromatograms showing the results of the qualitative analyses of rat brain and adrenal gland samples for (A) 2-MTHBC, (B) THBC, and (C) 6-MeOTHBC using selected ion monitoring (SIM) techniques. (Adrenal gland sample shown.)

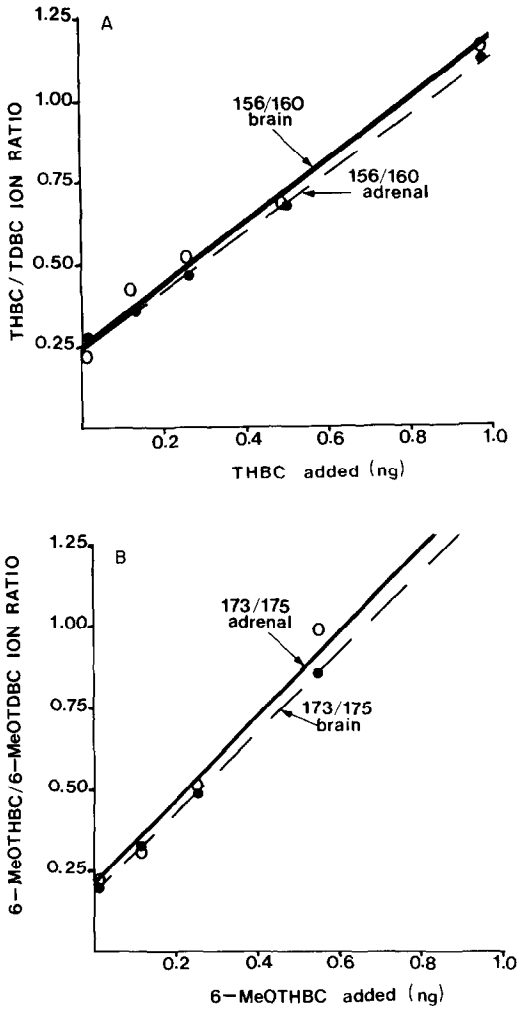

Fig. 6. Recovery curves for THBC (top panel) and 6-MeOTHBC (bottom panel) from pooled samples of rat brain and adrenal glands. The data were obtained by increasing the concentration of THBC and 6-MeOTHBC while holding the concentrations of TDBC and 6-MeOTDBC constant and measuring the ion (*m/e*) ratios for each compound respectively.

brain structures and to the greatest extent in the pineal. Further research on the localization of β-carbolines in discrete brain areas may provide additional support for these observations and may lead us to an understanding of their role *in vivo*.

Identification of 6-MeOTHBC and THBC in rat adrenal glands. The identification of 2-MTHBC, 6-MeOTHBC, and THBC in rat adrenal glands is a most interesting observation. Similar to the findings in brain tissues, 6-MeOTHBC appears to be present in the adrenal glands at approximately twice the concentration of THBC (Table 2). [Concentration/adrenal gland (0.066 g average weight) = 16.5 ng for THBC and 36.8 ng for 6-MeOTHBC.] While the precursors for the formation of THBC and 6-MeOTHBC have been identified in the adrenals of the rat, they are present at much lower levels (TA = 11.4 ± 2.5 ng/g [22]; 5-MeOTA = < 3.0 ng/g [23]). Thus, the possibility still exists that these β-carbolines may be sequestered in the adrenals following their formation in other areas of the body.

Possible mechanisms for the formation of β-carbolines in vivo. The apparent precursors for the formation of the β-carbolines identified in this study, TA [22], NMT [24], and 5-MeOTA [23], have all been identified as normal products of mammalian metabolism. Thus, THBC, 2-MTHBC, and 6-MeOTHBC may be formed non-enzymatically from the condensation of these indoleamines with formaldehyde. Honecker and Rommelspacher [20] have observed a 20-fold increase in THBC level in rat forebrain tissues following an intraperitoneal injection of TA (150 mg/kg). This dramatic increase in THBC may be due to increased condensation reactions of TA with formaldehyde, but an as yet unidentified enzymatic process for the formation of β-carbolines should not be ruled out. We have recently shown that 2-MTHBC and THBC are formed during the metabolism of *N,N*-dimethyltryptamine (DMT) or DMT-*N*-oxide, a major metabolite of DMT, in rat whole brain homogenates [25]. Although the addition of a formaldehyde trapping reagent to these incubations attenuated the formation of 2-MTHBC and THBC from DMT, it did not eliminate their production. This finding suggests that the formation of β-carbolines may occur by mechanisms other than those requiring the availability of formaldehyde in the free state. Mechanistically, intermediates in the formation of *β*-carbolines from an indoleamine and formaldehyde and those proposed in the demethylation of secondary and tertiary amines, via either direct *C*-hydroxylation or *N*-oxide rearrangement, are identical (Fig. 8). Thus, β-carbolines may also be formed *in vivo* from the enzymatic demethylation of secondary or tertiary indoleamines such as NMT [24], DMT [26], 5-methoxy-DMT [27, 28] or 5-hydroxy-DMT (bufotenin) [29–31]. Furthermore, the secondary amine *β*-carbolines, such as THBC, may also be formed by the demethylation of the tertiary amine *β*-carbolines, such as 2-MTHBC, or the secondary amine β-carbolines may be methylated to form the tertiary amine β-carbolines. The β-carbolines may also be derived from the formation of 3-carboxy-THBC, produced from the reaction of tryptophan with formaldehyde. Theoretically this compound could be decarboxylated, *N*-methylated, 6-hydroxylated and/or *O*-methylated to give rise to the *β*-carbolines identified in this study. Honecker and Rommelspacher, however, observed that a tryptophan load (150 mg/kg, i.p.) produced only a 2-fold increase in THBC in rat forebrain [20]. The fact that the same dosage of TA produced a 20-fold increase in THBC may indicate that the indoleamine provides a more direct and favorable route for the formation of the *β*-carbolines than the route involving the amino acid tryptophan.

Possible roles of THBC and 6-MeOTHBC in rat brain and adrenal gland. The presence of 2-MTHBC, THBC, and 6-MeOTHBC in rat brain and adrenal glands implies an as yet unidentified biochemical role for this family of compounds in these and perhaps other organs. THBC and 6-MeOTHBC have been shown to be potent inhibitors of 5-hydroxytryptamine (5-HT) neuronal uptake and to elevate

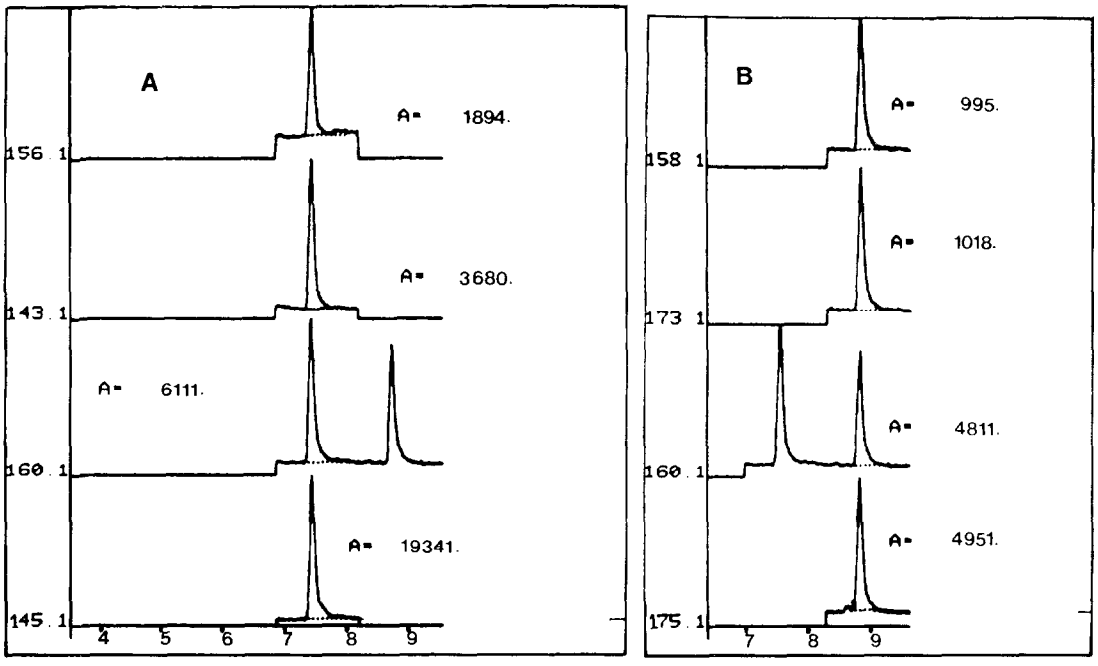

Fig. 7. Representative chromatograms showing the results of the quantitative analyses of rat brain and adrenal gland samples for (A) THBC and (B) 6-MeOTHBC using deuterated internal standards and selected ion monitoring (SIM) techniques. (Brain sample shown.)

plasma and brain levels of 5-HT [32–39]. These compounds are also inhibitors of monoamine oxidase (MAO) [33, 40]. Recent work has also shown that THBC has an exceedingly potent effect on alcohol consumption in the rat [41].

In 1961 McIsaac [42] described a factor in pineal tissue that was believed to be 1-methyl-6-MeOTHBC. Submicrogram quantities of this compound were shown to produce a maximal secretion of aldosterone without affecting levels of cortisol. Similarly, Meyer and Buckholtz [43] have observed that 6-MeOTHBC causes an increase in corticosterone secretion in mice, indicative of enhanced pituitary–adrenal axis activity. Thus, β-carbolines may be involved in stress modulation. Their apparent storage in adrenal glands may provide a mechanism for their release during stressful situations to exert hormone-like effects. Localization of the β-carbo-

Table 2. Quantitative analyses of rat brain and adrenal glands for THBC and 6-MeOTHBC

Sample	Tissue wt (g)	Ion ratio (*m/e*) THBC/TDBC (156/160)	Ion ratio (*m/e*) 6-MeOTHBC/6-MeOTDBC (173/175)	THBC (ng/g wet wt)	6-MeOTHBC (ng/g wet wt)
Brain					
1	2.103	0.147	0.0893	11.6	22.9
2	2.208	0.294	0.1152	22.2	28.2
3	2.183	0.175	0.2057	13.3	50.9
4	2.150		0.2464		61.9
5	2.247				
6	2.084	0.310	0.1049	24.0	27.2
7	2.236	0.232	0.0937	16.4	22.6
			Mean ± S.D. =	17.5 ± 4.86	35.6 ± 16.6
Adrenal					
1	0.088	0.160	0.196	302.2	924.8
2	0.071	0.180		422.5	
3	0.045	0.214	0.154	791.1	1168.2
4	0.058	0.154	0.114	441.5	1064.2
5	0.075	0.196	0.114	434.7	817.2
6	0.082	0.230		467.1	
7	0.044	0.171	0.130	642.7	1594.0
			Mean ± S.D. =	500.3 ± 163	1113.7 ± 300

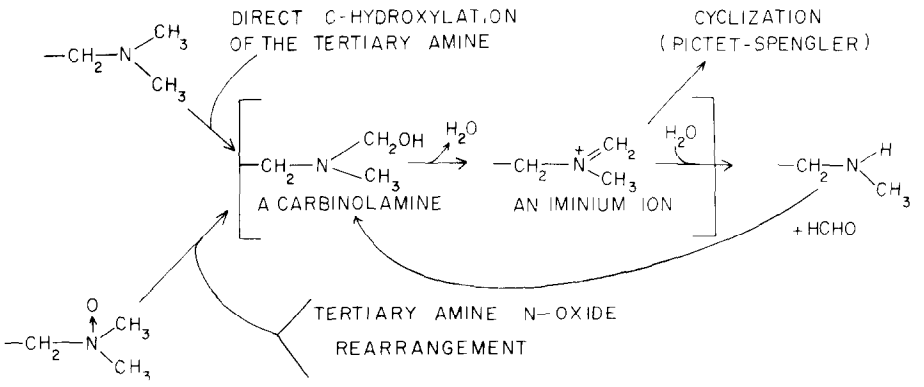

Fig. 8. Mechanisms for the demethylation of tertiary amines and tertiary amine *N*-oxides illustrating the intermediates that are identical with those proposed in the Pictet–Spengler reaction.

lines in either the adrenal medulla or cortex may provide further insight into their possible role in stress.

In conclusion, the endogenous β-carbolines, given their known pharmacological effects, may be a new class of hormonal or neuromodulatory compounds.

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