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D. DE BOER, L.J.A.L.DOS REYS, I.J. BOSMAN, R.A.A. MAES:
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The detection of the use of some popular hallucinogenic compounds, including those of *Psilocybe* mushrooms

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Introduction

In the sixties and seventies the abuse of hallucinogenic compounds in the Western World was considered to be a significant problem. Nowadays these types of drugs have made a comeback. Again there seems to be a close association with the evolution of certain music trends. Like for the amphetamine designer drugs [1], athletes may use these types of drugs for recreational reasons. This overview gives information regarding the implementation of the detection of the abuse of some current hallucinogenic drugs by the GC/MS analytical doping control procedure for stimulants, narcotics and beta-blockers after *N*-TFA-*O*-TMS derivatisation [2].

Classification

The respective compounds can be divided into two structurally distinct groups, namely the mescaline- (I) and tryptamine-like (II) compounds (Figure 1).

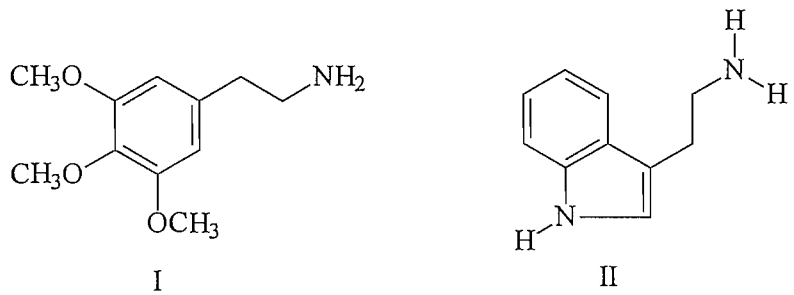
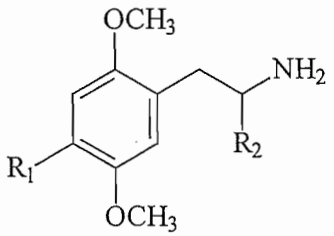


Figure 1 Structures of some basic hallucinogenic compounds: mescaline- (I) and tryptamine (II)

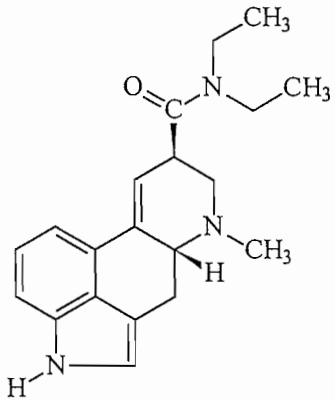
Famous examples of hallucinogenic drugs of the mescaline-like group (Figure 2) of the sixties and seventies are DOM (IIIa; 4-methyl-2,5-dimethoxyamphetamine) and DOB (IIIb; 4-bromo-2,5-dimethoxyamphetamine). Of the tryptamine-like group (Figure 3) it is of course LSD (IV; lysergic acid diethylamide).

Nowadays other kind of examples are more popular, namely of the mescaline-like group (Figure 2) 2C-B (IIIc; 4-bromo-2,5-dimethoxyphenethylamine) and 2C-T-2 (IIId; 4-ethylthio-2,5-dimethoxyphenethylamine) and of the tryptamine-like group (Figure 3) psilocin (Va) and psilocybin (Vb). While 2C-B and 2C-T-2 are pure synthetic compounds, psilocin and psilocybin are natural alkaloids. These alkaloids originate from amongst others *Psilocybe* mushrooms, which are also known as "magic" mushrooms [3]. Probably, psilocin is in mushrooms a decomposition product of psilocybin, which is found either in the mushroom itself or is formed during chemical isolation of psilocybin.

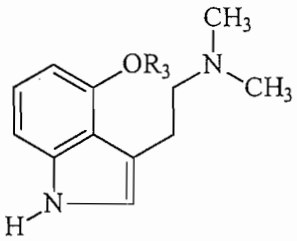


IIIa DOM $R_1 = CH_3$; $R_2 = CH_3$
IIIb DOB $R_1 = Br$; $R_2 = CH_3$
IIIc 2C-B $R_1 = Br$; $R_2 = H$
IIId 2C-T-2 $R_1 = CH_3CH_2S$; $R_2 = H$

Figure 2 Structures of some ancient and current popular mescaline-like compounds



IV

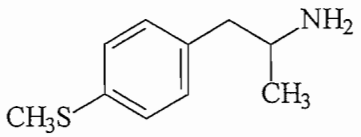


Va $R_3 = H$
Vb $R_3 = PO_3$

Figure 3 Structures of some ancient and current popular tryptamine-like compounds: LSD (IV), psilocin (Va) and psilocybin (Vb).

Regarding the classification of hallucinogenic drugs in a mescaline- and tryptamine-like group there is one exception worth mentioning, namely *para*-methylthioamphetamine (Figure 4; 4-MTA). This compound is sold on the same segment of market of hallucinogenic

drugs, but has more an amphetamine-like action. It is a strong serotonin releasing compound and because of that can relatively easily cause intoxications [4,5].



VI

Figure 4 Structure of 4-MTA (VI)

Metabolism

In respect to the metabolism of the current popular mescaline-like compounds, the available information in scientific literature is limited. Mescaline itself has been studied in the sixties and undergoes oxidative deamination to form benzoic acid- and acetic acid-like [6]. Other steps include *N*-acetylation and demethylation of methoxy-groups. The different steps can also be combined, resulting in a metabolite as for example *N*-acetyl-3,4-dimethoxy-5-hydroxyphenethylamine. It can be assumed that 2C-B and 2C-T-2 will metabolise in similar ways. An *in vitro* study confirmed this assumption for 2C-B (Figure 5) [7]. Actually, for 2C-B a preliminary *in vivo* study also has been performed [9]. The presence of the parent compound and of 4-bromo-2,5-dimethoxyphenylacetic acid (metabolite 5) in the urine specimen of an abuser was found and confirmed using reference standards. Furthermore the formation of 4-bromo-2,5-dimethoxybenzoic acid (metabolite 4) and of 4-bromo-5-hydroxy-2-methoxyphenethylamine (metabolite 3) was indicated by GC/MS. Of these last two metabolites no reference standards were available. Despite of the possible metabolic steps involved in the biotransformation of mescaline-like compounds, it can be concluded at this stage that probably at least the respective parent compounds can be detected after intake of such substances.

The tryptamine-like compounds psilocin and psilicybin have been studied in sufficient details [8] to conclude that psilocin is the metabolite of interest if one would like to screen for the abuse of *Psilocybe* mushrooms. Because psilocin is partly excreted conjugated, a hydrolysis step is required for optimal recovery of this metabolite.

The metabolism of 4-MTA has not been studied yet, although it can be expected that it will be similar as that of amphetamine with the exception of possible metabolites with a

modified methylthio-group. In an *in vitro* study in which 4-MTA was incubated in an NADPH generating system with rat liver homogenates, the formation of 4-methylthiobenzoic acid was observed [10]. In the reported intoxication studies the parent compound was always identified [5].

Target compounds and the analytical doping control procedure

For identification purposes the GC/MS analytical doping control procedure for stimulants, narcotics and beta-blockers after *N*-TFA-*O*-TMS derivatisation [2] is the method of first choice. Considering the known and possible metabolism of the compounds of interest it could be concluded that at least the parent compounds 2C-B, 2C-T-2 and 4-MTA are the target compounds of interest for the detection of their abuse. Using the method of choice *N*-TFA derivatives of the respective compounds will be obtained. For "magic" mushrooms the target compound is psilocin. However, the expected *N*-TFA-*O*-TMS derivative of psilocin was not obtained using the method studied. A method using a derivatisation reaction with MSTFA instead of MSTFA combined with MBTFA proved to produce better results. That way the *N*-TMS-*O*-TMS derivative was acquired. The relevant mass spectra are shown in the Appendix.

Conclusions

1. Besides the parent compounds some metabolites may be found in urine after the abuse of the current popular hallucinogenic drugs.

2. The analytical procedures should be focussed for the time being on parent compounds, except for "magic" mushrooms for which psilocin should be the target compound.

3. Using the GC/MS analytical doping control procedure for stimulants, narcotics and beta-blockers with *N*-TFA-*O*-TMS derivatisation, the parent compounds of the current popular hallucinogenic drugs will be converted into *N*-TFA derivatives. The detection of psilocin requires a modified derivatisation procedure.

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10 Not published *in vitro* study of the incubation of 4-MTA in NADPH generating system with rat liver homogenate.

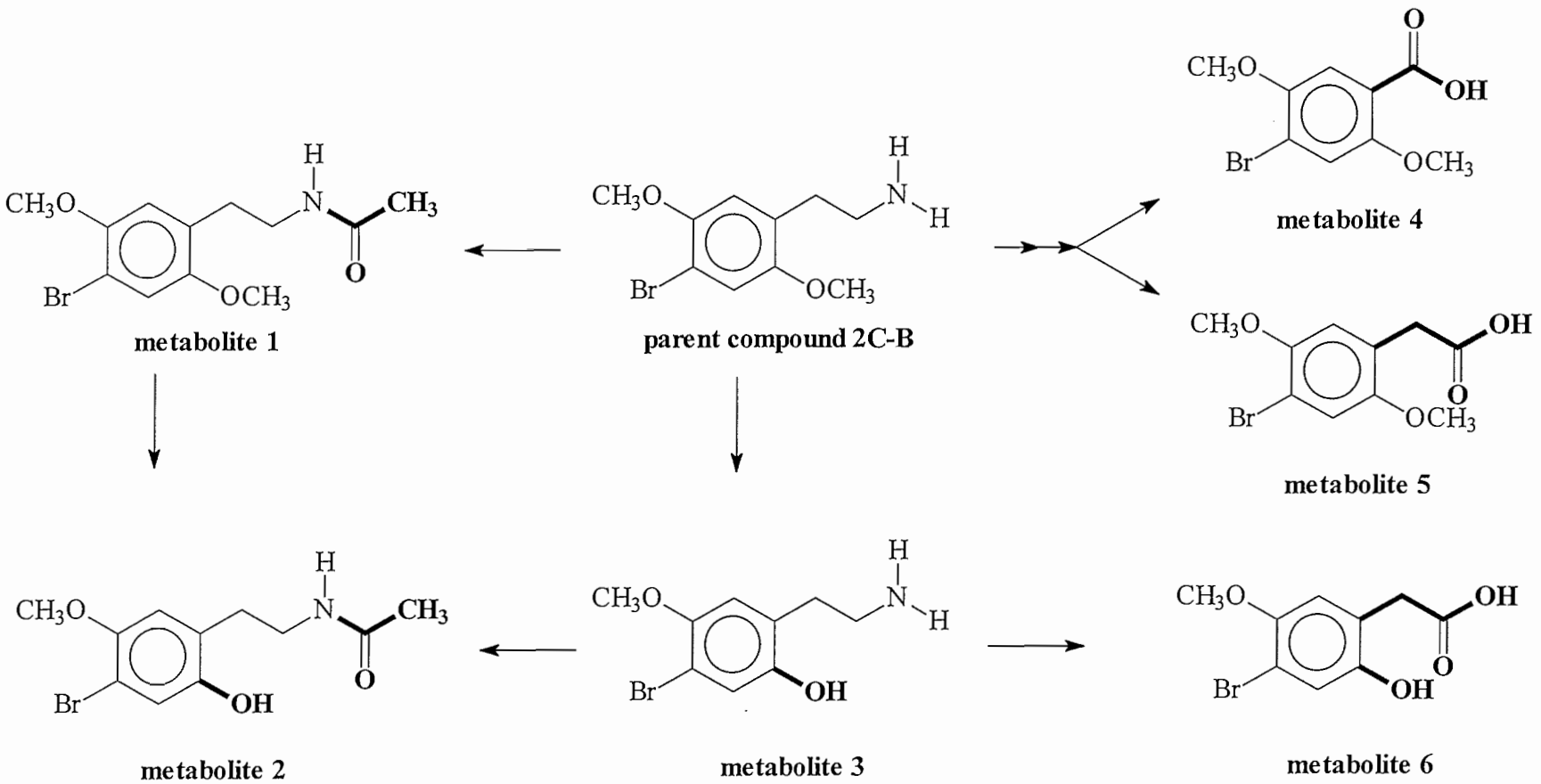
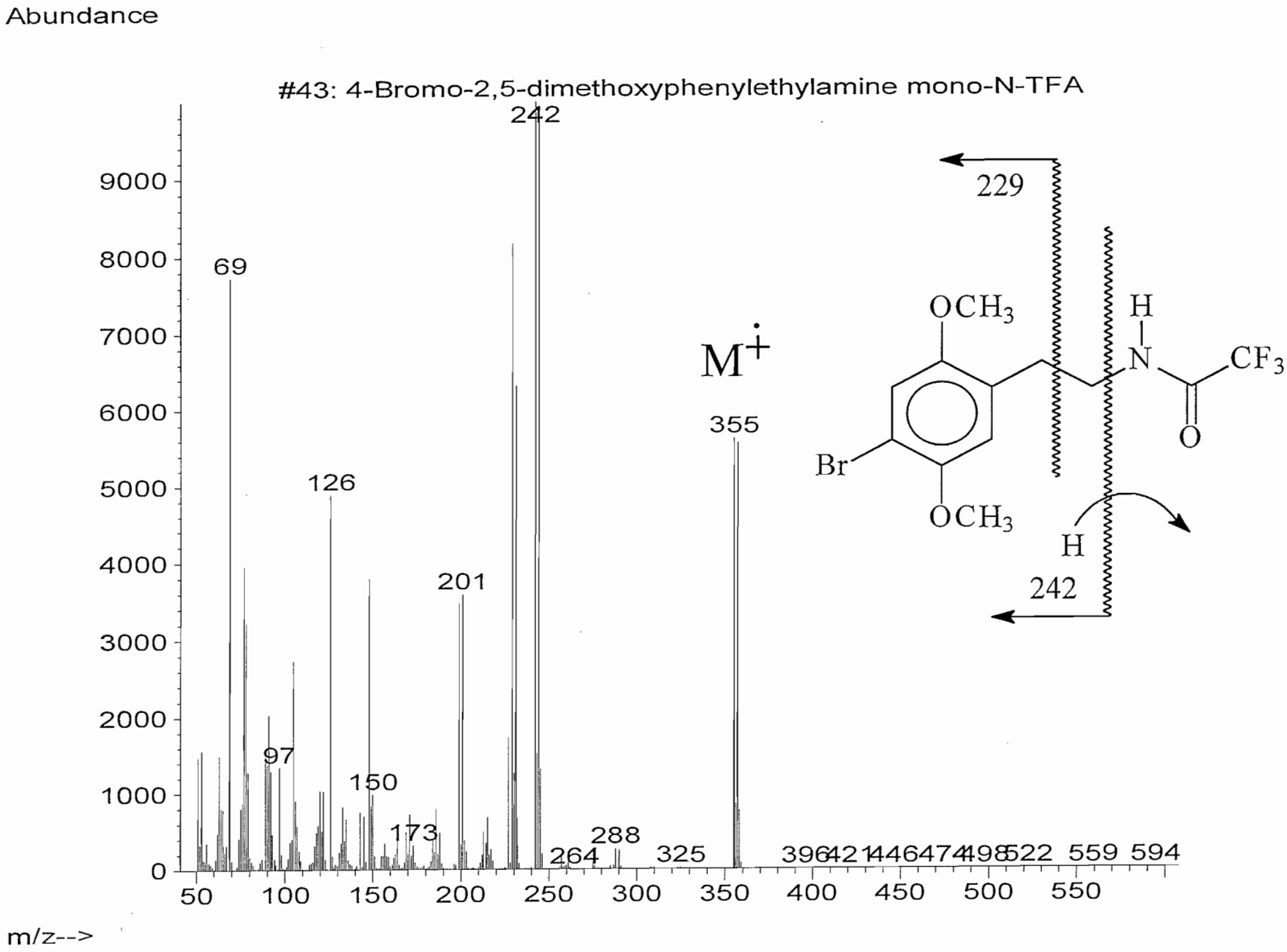
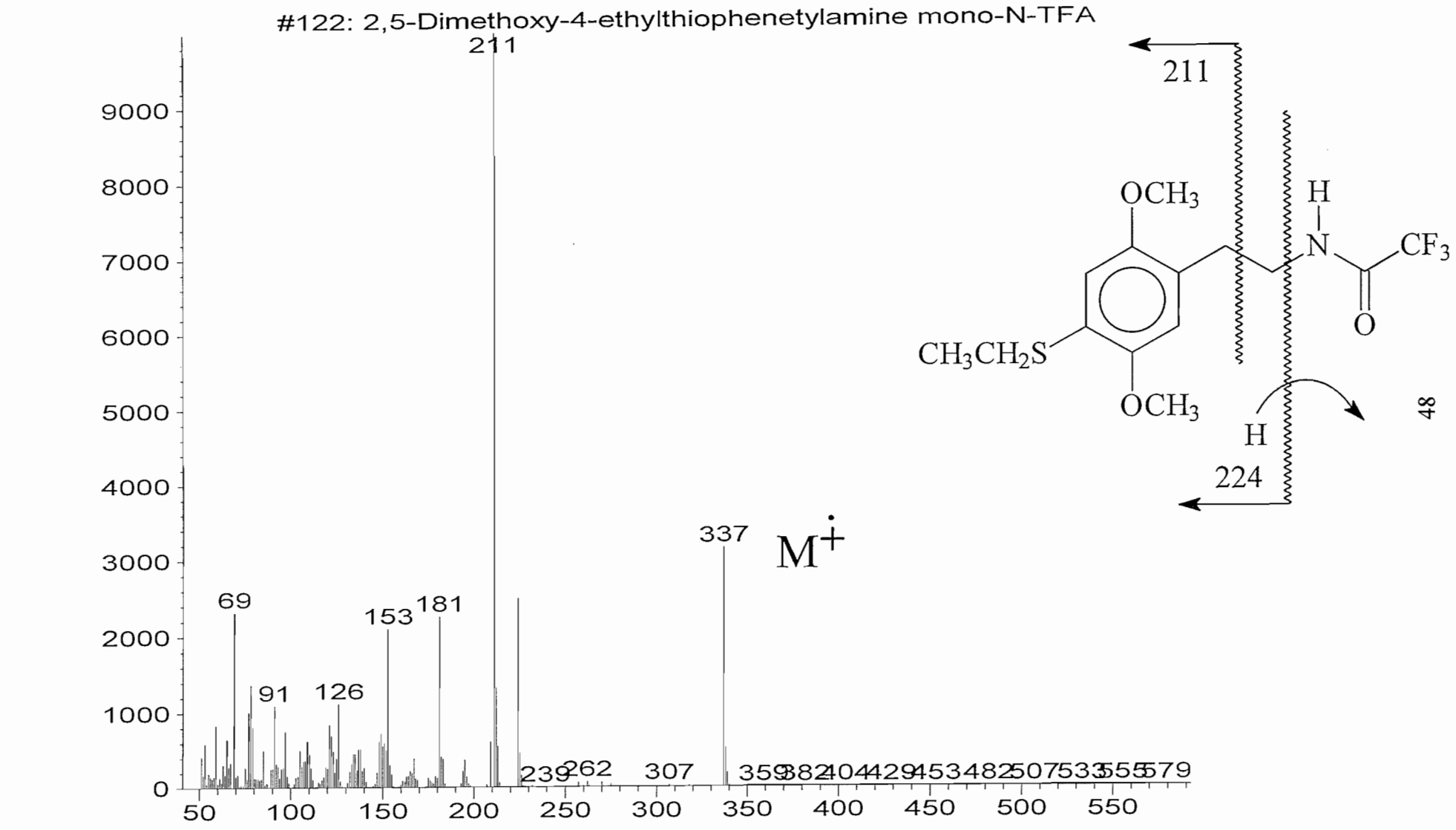
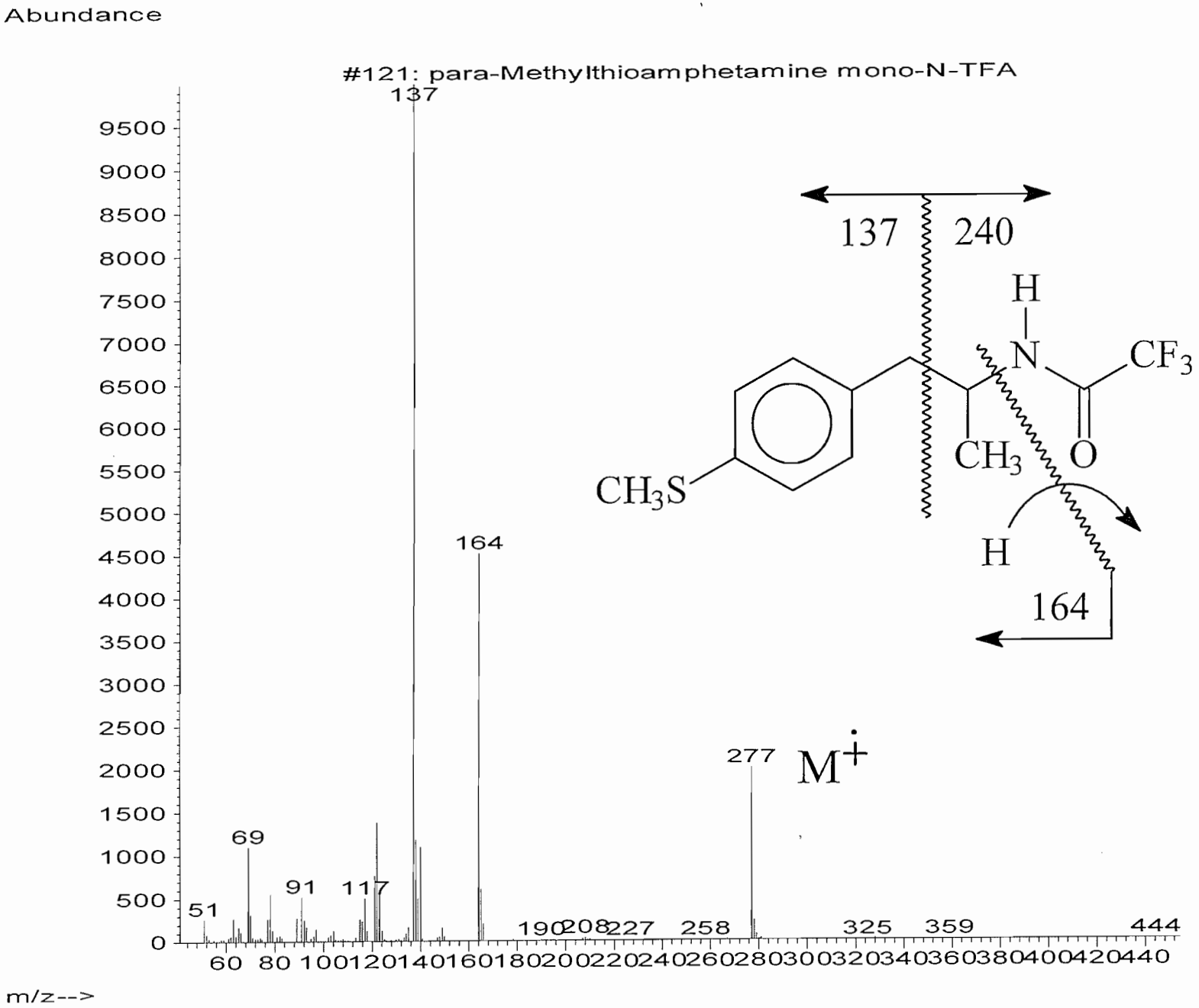
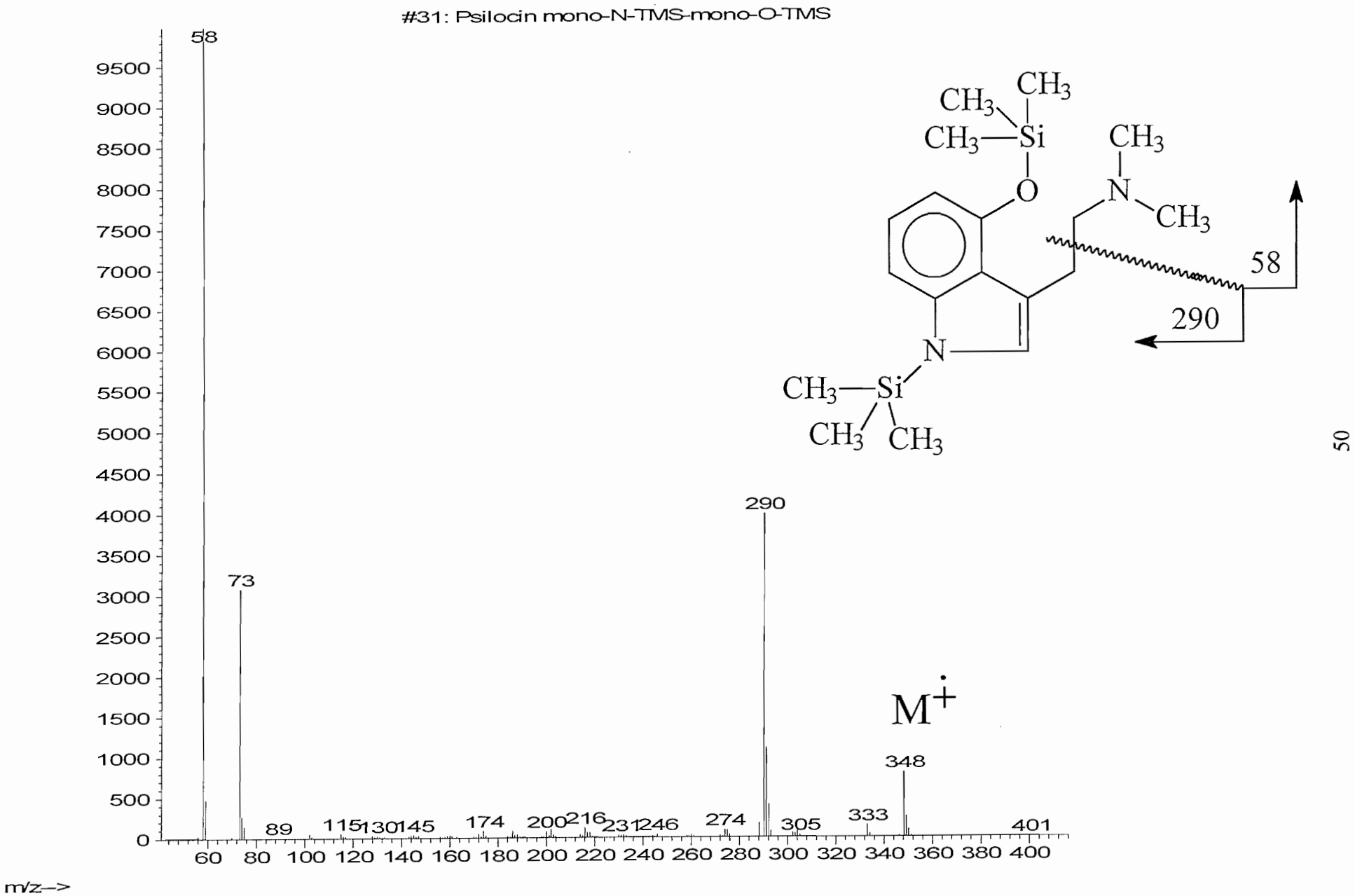


Figure 2 Metabolites observed after incubation of 2C-B in NADPH generating system with rat liver homogenate. In bold the site of biotransformation is indicated [7].


Abundance
#43: 4-Bromo-2,5-dimethoxyphenylethylamine mono-N-TFA
242
69
126
201
97
150
173
288
264
325
355
396
421
446
474
498
522
559
594
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
50
100
150
200
250
300
350
400
450
500
550
m/z-->
M+
229
242
OCH3
OCH3
Br
H
N
CF3
O
H


Abundance
#122: 2,5-Dimethoxy-4-ethylthiophenetylamine mono-N-TFA
211
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
69
91
126
153
181
239
262
307
337
M+
359
382
404
429
453
482
507
533
555
579
50
100
150
200
250
300
350
400
450
500
550
m/z-->
211
OCH3
H
N
CF3
O
CH3CH2S
OCH3
H
224
48


Abundance
#121: para-Methylthioamphetamine mono-N-TFA
137
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
164
277
M+
69
91
117
51
190
208
227
258
325
359
444
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440
m/z-->
137
240
H
N
CF3
CH3S
CH3
O
H
164


Abundance
#31: Psilocin mono-N-TMS-mono-O-TMS
58
73
290
348
M+
89
115
130
145
174
200
216
231
246
274
305
333
401
9500
9000
8500
8000
7500
7000
6500
6000
5500
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400
m/z-->
CH3
CH3
CH3
Si
O
CH3
N
CH3
58
290
N
CH3
Si
CH3
CH3
50