GLC–Mass Spectral Determination of Mescaline in Plasma of Rabbits after Intravenous Injection

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Abstract □ A GLC–mass spectral analysis with a deuterated internal standard was developed to measure plasma mescaline concentrations after intravenous administration to rabbits. The drug and the internal standard were extracted with benzene, derivatized with trifluoroacetic acid anhydride, and chromatographed on 2.5% QF-1 with mass fragmentographic detection. The detection limit is 5 ng/ml of plasma. The relative standard deviation was ~5%. The main advantage of this method is that it combines the specificity of the GLC retention time and mass spectral fragmentation pattern with the sensitivity of the mass fragmentographic detection.

Keyphrases □ Mescaline— GLC–mass spectral determination, rabbit plasma, intravenous injection □ Hallucinogenics—mescaline, GLC–mass spectral determination, rabbit plasma, intravenous injection □ GLC–mass spectrometry—analysis, mescaline in rabbit plasma

Mescaline (3,4,5-trimethoxyphenethylamine), an alkaloid isolated in 1896 from the peyote cactus *Lophophora Williamsii*, causes unusual psychic effects and visual hallucinations. In the United States, it is not considered a narcotic drug; but in most countries, it is on the list of illicit drugs. A review of its chemistry, biogenesis, and biological effects was published previously (1).

In clinical toxicological laboratories, identification and quantitation of mescaline are sometimes requested. Only a few methods relate to biological samples (2–4). Most references concern systematic mescaline identification by combined TLC (5–7), GLC and/or mass spectrometry (8–10), or high-pressure liquid chromatography (11). A radioimmunological method also was described (12).

Mescaline is excreted primarily in the urine in humans: subjects given 500 mg po excreted 87% of the dose in 24 hr. The unchanged compound accounted for 60%, and 3,4,5-trimethoxyphenylacetic acid accounted for ~30% (13).

This paper reports a specific and sensitive method for the quantitative determination of mescaline as trifluoroacetylmescaline (I) with trifluoroacetyl-$^2H_2$-mescaline (II) as the internal standard.

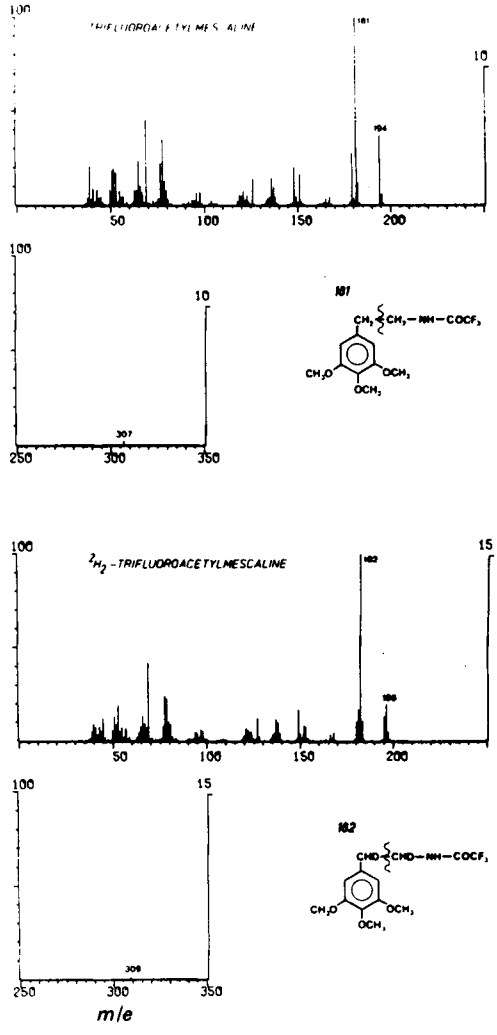

Figure 1—*The 70-ev electron-impact mass spectra of I (top) and II (bottom).*

EXPERIMENTAL

Materials—All chemicals were analytical reagent grade and used as received. Mescaline[1] was 99% pure. ^2H-Labeled mescaline was synthesized from 3,4,5-trimethoxybenzaldehyde by condensation with nitromethane and subsequent reduction with lithium aluminum deuteride. All labeled and nonlabeled standards were prepared in methanol at concentrations permitting use of 10–50-μl aliquots.

Table I—Influence of Overnight Storage Temperature (4 or −18°) on Plasma Mescaline Contents

Sample	Corrected Areas Ratio: Storage at 4°	Corrected Areas Ratio: Storage at −18°
1	0.416	0.417
2	0.443	0.455
3	0.441	0.458
4	0.464	0.471
5	0.457	0.487
6	0.444	0.460
$\bar{x}$	0.444	0.458
σ	0.017	0.023
CV	3.7%	5.1%

Subjects—Rabbits, 2 ± 0.1 kg, were given 1 ml of an aqueous solution of mescaline at a 10-mg/kg dose by rapid intravenous injection in the ear. Blood (1 ml) was drawn from the other ear and collected in heparinized tubes. The samples were centrifuged, and the plasma was stored at 4° until analysis on the next day.

Apparatus—Determinations were performed by GLC[2] with a mass spectrometer as a specific detector[3] coupled to a data system[4]. A silanized

[1] Koch and Light Laboratories.
[2] Varian model 1400.
[3] Finnigan model 3000.
[4] Finnigan model 6000 interactive data system.

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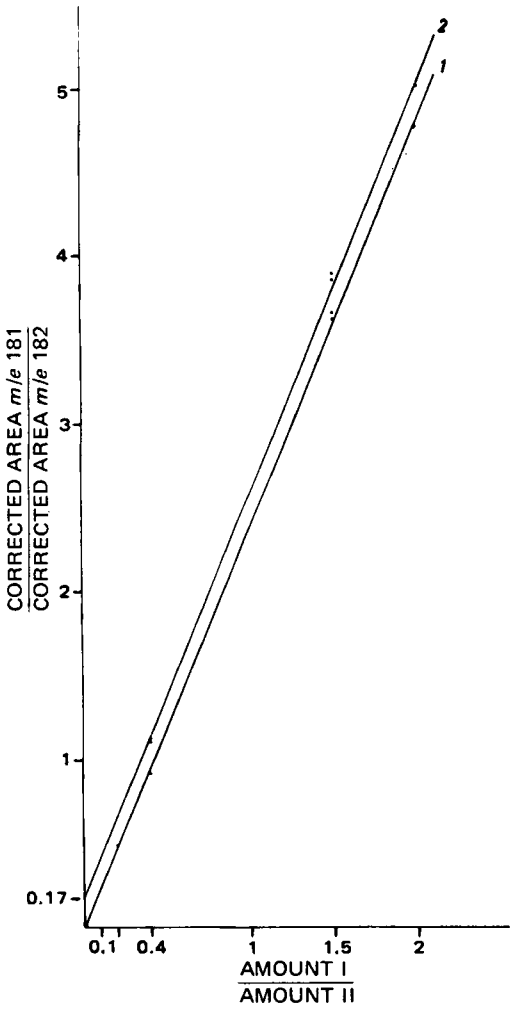

Figure 2—*Calibration graphs after correction for mutual contribution (1) and for the contribution of I to II (2) only.*

glass column, 1.5 m × 0.63 cm, was packed with 2.5% QF-1 on 100–120-mesh Varaport. Helium carrier gas flow was maintained at 30–35 ml/min. Injection port and oven temperatures were 210 and 195°, respectively. The mass spectrometer was operated at a pressure of 5×10^{-6} torr; the ionizing energy in the ion source was 70 ev.

Extraction—To 0.5 ml of plasma was added 20 μl of internal standard solution (200 ng of II). The pH was adjusted to 10 with 1 ml of ammonia–ammonium chloride buffer. The sample was then extracted with 4 ml of benzene. The extracts were dried over anhydrous sodium sulfate (~2 g) and evaporated to dryness under nitrogen.

The residue was dissolved in 0.2 ml of ethyl acetate and 0.2 ml of trifluoroacetic acid anhydride. The solution was allowed to react at 60° in a warm water bath for 30 min, after which the solvents were evaporated with a nitrogen stream. Then the residue was dissolved in 10–20 μl of methanol, and 1–2-μl aliquots were analyzed. The retention time for mescaline and the internal standard was 2.60 min.

Quantitation—Standard solutions in methanol, containing quantities equivalent to 20, 40, 80, 200, and 400 ng of mescaline/μl and 200 ng of internal standard/μl, were used to establish the calibration curve.

RESULTS AND DISCUSSION

The mass spectra of mescaline and of the internal standard are shown in Fig. 1. From the intensity ratio of the *m/e* 181 and 182 ions, the plasma concentrations could be calculated. The problem was that the *m/e* 181 ion in the mescaline spectrum had an isotopic peak at *m/e* 182, while the fragmentation pattern of the internal standard showed both *m/e* 181 and 182 peaks. Consequently, the standard calibration graph shows a curved line with an intercept different from zero, depending on the relative intensity of the *m/e* 181 ion to the *m/e* 182 ion in the mass spectrum of the internal standard. However, this problem could be overcome by correcting

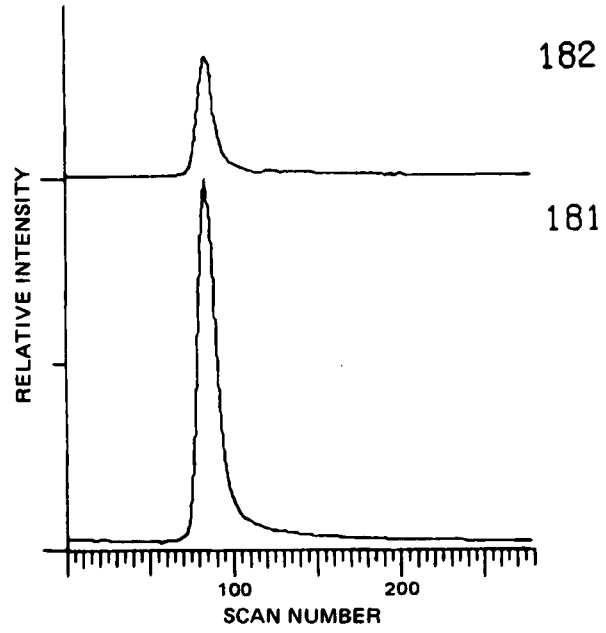

Figure 3—*Mass fragmentogram of a plasma extract (*m/e *181 = trace of I, and* m/e *182 = trace of II).*

for those mutual interferences using:

$$S_{181} = (f_x)(C_x) + (a)(f_s)(C_s) \quad \text{(Eq. 1)}$$

$$S_{182} = (f_s)(C_s) + (b)(f_x)(C_x) \quad \text{(Eq. 2)}$$

where:

C_x = amount of mescaline present in the extract
f_x = factor relating concentration to intensity of the *m/e* 181 ion in the mescaline spectrum
C_s = amount of internal standard in the extract
f_s = factor relating concentration to intensity of the *m/e* 182 ion in the internal standard spectrum
a = relative intensity of the *m/e* 181 ion to the *m/e* 182 ion in the mass spectrum of the internal standard
b = relative intensity of the *m/e* 182 ion to the *m/e* 181 ion in the mescaline mass spectrum
S_{181} = integrated area of the 181 channel
S_{182} = integrated area of the 182 channel

Equations 1 and 2 can be rewritten as:

$$\frac{S_{181} - (a)(S_{182})}{S_{182} - (b)(S_{181})} = (A)(C_x) \quad \text{(Eq. 3)}$$

where:

$$A = \left(\frac{f_x}{f_s}\right)\left(\frac{1}{C_s}\right) \quad \text{(Eq. 4)}$$

The ratio of the corrected areas is now directly proportional to the concentration of the unknown in the standard (Fig. 2). Curve 1 is calculated

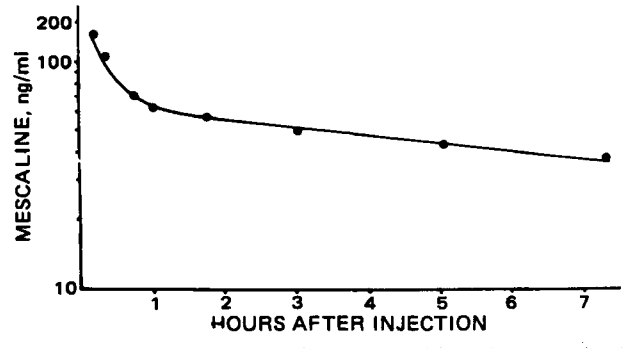

Figure 4—*Profile curve of mescaline in one subject after 10 mg/kg iv.*

by linear regression from the experimental data with $a = 0.165$ and $b = 0.105$. The latter values are obtained by injecting standard solutions of I and II and measuring the ratios of *m/e* 182 to 181 and of *m/e* 181 to 182, respectively. The equation is given by:

$$y = 0.05x + 0.0013 \qquad \text{(Eq. 5)}$$

where y is the corrected areas ratio and x is the mescaline concentration.

Curve 2 is calculated with $a = 0$ and $b = 0.105$ when no corrections are made for the constant contribution of the internal standard's *m/e* 181 ion to the compound to be measured. In this case, the intercept is 0.17, which is in reasonably good agreement with the expected value of 0.165.

An example of a mass fragmentogram of a plasma extract is shown in Fig. 3. No coeluting substances were found when blank plasma samples were analyzed. Therefore, the method, due to the combined specificity of both retention time and mass spectrometric fragmentation pattern, is suitable for pharmacokinetic studies.

The influence of the overnight storage temperature was examined by analyzing a 0.05-ppm spiked pool serum sample, part of which had been kept at 4° and another part at −18° overnight (Table I). The mean values ($n = 6$) of each set of assays were not significantly different (Student *t* test).

The precision of this analytical procedure, expressed as the coefficient of variation, is on the order of 5%. For the GLC–mass spectral quantitative assay, it was 1.7% (six repetitive injections of the same extract).

Although the apparent extraction efficiency with a labeled internal standard added before the extraction was 100%, the actual efficiency was checked; it was about 95% for spiked serum samples. To illustrate that the method is suitable for biological profile studies, the profile curve of one subject is shown in Fig. 4.

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New Compounds: Reissert Compound Studies XXXII: Facile Synthesis of 3-Azapapaverine

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Received June 18, 1979, from the *Department of Chemistry, University of Missouri–Kansas City, Kansas City, MO 64110.* Accepted for publication July 31, 1979.

Abstract □ 6,7-Dimethoxyphthalazine was obtained in four steps from veratric acid and converted to its Reissert compound. Alkylation of this Reissert compound with 3,4-dimethoxybenzyl chloride followed by hydrolysis gave 3-azapapaverine.

Keyphrases □ Reissert compounds—synthesis of 3-azapapaverine from veratric acid □ 3-Azapapaverine—synthesis from veratric acid *via* a Reissert compound □ Phthalazines—synthesis of 3-azapapaverine from veratric acid *via* Reissert compound derivative of 6,7-dimethoxyphthalazine

The application of Reissert compounds (1, 2) to the synthesis of natural products and compounds of potential medicinal interest is well documented (2, 3) in the field of quinolines and, particularly, isoquinolines. Although phthalazine also yields a Reissert compound (4, 5), its use in the synthesis of potential medicinal agents has not been studied. This paper reports a convenient synthesis of 3-azapapaverine (I) using the Reissert compound (II) of 6,7-dimethoxyphthalazine (III) and demonstrates the potential utility of Reissert compounds derived from phthalazine.

DISCUSSION

A convenient synthesis of III from commercially available veratric acid (IV) was developed using modifications of several literature procedures. Chloromethylation of IV gave the lactone (V) (6), which yielded 4,5-dimethoxyphthalyl alcohol (VI) on reduction with lithium aluminum hydride (7). Oxidation of VI with activated manganese dioxide gave 4,5-dimethoxyphthalaldehyde (VII). Treatment of this dialdehyde (VII) with hydrazine hydrate gave III (8). The overall yield of III from IV was 36%.

Compound III was converted to its Reissert compound (II) by a phase transfer procedure (5) or by a trimethylsilyl cyanide procedure (9). Alkylation of II with 3,4-dimethoxybenzyl chloride in the presence of sodium hydride gave the alkylated Reissert compound VIII. Hydrolysis of VIII with potassium hydroxide gave I.

This procedure should be valuable in the synthesis of phthalazine analogs of isoquinolines of medicinal interest.

EXPERIMENTAL

***m*-Meconin (V)**—Formaldehyde (230 ml, 37% solution) was saturated with hydrogen chloride gas at 15–20°, and 32 g (0.176 mole) of IV[1] was added. The mixture was heated to 60–70° for 7 hr, during which hydrogen chloride gas was bubbled slowly through it. The mixture was cooled, kept overnight, and concentrated *in vacuo*. Water, 100 ml, was added, and the mixture was neutralized with 2:3 dilute ammonium hydroxide. A solid formed and was filtered, washed several times with water, and dried. Recrystallization from methanol gave 22.4 g (65.6%) of V, mp 154–155° [lit. (6) mp 154–156°]; IR (KBr): 3080, 3020 (m), 2940 (w), 1755–1740 (s), 1600 (s), 1500 (s), 1350 (s), and 1300 (s) cm^{-1}.

4,5-Dimethoxyphthalyl Alcohol (VI)—A warm solution of 14 g (0.072 mole) of V in 150 ml of anhydrous tetrahydrofuran was added continuously to a suspension of 7.5 g of lithium aluminum hydride in 80 ml of tetrahydrofuran, and the mixture was refluxed for 4 hr. The mixture was cooled in an ice bath, and the excess lithium aluminum hydride was

[1] Aldrich.

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