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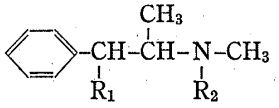
Preparation of a Specific Antibody to Methamphetamine

N-Carboxymethylmethamphetamine(III) has been synthesized directly from methamphetamine(I) and through a new route starting from ephedrine(IV). This new hapten was conjugated with BSA and the antiserum for I was prepared by immunization of rabbits with the conjugate (VIII). The production of the antibody for I was confirmed by the ring test and Ouchterlony method.

Keywords——radioimmunoassay; methamphetamine; new synthetic route; N-carboxymethylmethamphetamine; N-carboxymethylmethamphetamine-BSA; specific antibody; antiserum; ouchterlony method

Methamphetamine is widely noted for the distinctive antihypnotic activity. Since this medicine has often caused a serious social problem owing to its dangerous abuse, the preparation, storage and consumption of the antihypnotics have always been under a severe legal restriction. This particular situation has led us first to device a new synthetic method of N-carboxymethylmethamphetamine (III) being derived from readily available ephedrine(IV) without use of methamphetamine (I), which is also prepared directly from I. The present paper concerns the preparation of III, the conjugate of the hapten with bovine serum albumin (BSA) (VIII) and the specific antibody being utilized for the radioimmunoassay for I, which will be described in the following paper.



I: R_1=H, R_2=H
II: R_1=H, R_2=$CH_2CO_2CH_2CH_3$
III: R_1=H, R_2=CH_2CO_2H
IV: R_1=OH, R_2=H
V: R_1=Cl, R_2=H
VI: R_1=SC_6H_5, R_2=H
VII: R_1=SC_6H_5, R_2=$CH_2CO_2CH_2CH_3$
VIII: R_1=H, R_2=CH_2CONH-BSA
IX: R_1=H, R_2=CH_2CONH-EA[a]

a) EA=egg albumin

Chart 1. Diagram of the Formulas of Methamphetamine Derivatives

Treatment of I in benzene with ethyl bromoacetate in the presence of anhydrous sodium carbonate (Na_2CO_3) at room temperature overnight afforded, in 71% of yield, 1-phenyl-2-(N-ethoxycarbonylmethyl-N-methyl)aminopropane(II) as a colorless oil. $C_{14}H_{21}O_2N$.[1] Mass Spectrum *m/e*: 236 (QM^+).[2] bp 151—154° (5 mm Hg). IR ν_{max}^{KBr}: 1753 cm^{-1} (C=O). PMR

1) Satisfactory elemental analyses were obtained for all the new compounds described in this paper.
2) Quasi molecular ion observed by the chemical ionization mass spectrometry using methane as a reagent gas.

(in $CDCl_3$) δppm: 0.94 (3H, d 6, >CH–C<u>H</u>$_3$), 1.26 (3H, t 7, –CO_2CH_2C<u>H</u>$_3$), 2.45 (3H, s, >N–C<u>H</u>$_3$), 2.45 (1H, m, >C<u>H</u>–CH_3), 3.00 (2H, m, C_6H_5C<u>H</u>$_2$–), 3.32 (2H, s, >N–C<u>H</u>$_2CO_2$–), 4.20 (2H, quartet 7, –CO_2C<u>H</u>$_2CH_3$), 7.22 (5H, m, aromatic–<u>H</u>). Hydrolysis of II with 5% potassium hydroxide(KOH)–methanol(MeOH) furnished, in 79% of yield, N-carboxymethylmethamphetamine (III) as colorless prisms. $C_{12}H_{17}O_2N$. mp 142.5—143.5°. Mass Spectrum *m/e*: 208 (QM$^+$). IR ν_{max}^{KBr}: 1620 cm^{-1} (C=O). PMR (in $CDCl_3$) δppm: 1.19 (3H, d 6, >CH–C<u>H</u>$_3$), 2.93 (3H, s, >N–C<u>H</u>$_3$), 3.00 (2H, m, C_6H_5C<u>H</u>$_2$–), 3.73 (2H, s, >N–C<u>H</u>$_2CO_2$–), 3.73 (1H, m, >C<u>H</u>–CH_3), 7.28 (5H, m, aromatic–<u>H</u>).

A new route of preparation of III is described as follows. A solution of pseudochloroephedrine (V) prepared from *dl*-ephedrine (IV) and sodium thiophenolate in tetrahydrofuran(THF)-ethanol(EtOH) was allowed to stand at room temperature for 10 hr furnishing 1-phenyl-1-phenylthio-2-methylaminopropane (VI) in 35—50% of yield. $C_{16}H_{19}NS\cdot HCl$. mp 183—184°. Mass Spectrum *m/e*: 258 (QM$^+$). PMR (in $CDCl_3$) δppm: 1.26 (3H, d 6, >CH–C<u>H</u>$_3$), 2.69 (3H, s, >N–C<u>H</u>$_3$), 3.45 (1H, m, >CH–C<u>H</u>$_3$), 4.51 (1H, d 8, C_6H_5S–C<u>H</u>–CH<), 7.28 (10H, m, aromatic-<u>H</u>). 1-Phenyl-1-phenylthio-2-(N-ethoxycarbonylmethyl-N-methyl)aminopropane (VII) was obtained in 86% of yield by treatment of VI with ethyl bromoacetate and Na_2CO_3 at room temperature for a day. $C_{20}H_{25}O_2NS\cdot 0.5H_2PtCl_6$. mp 111—113°. Mass Spectrum *m/e*: 344 (QM$^+$). IR ν_{max}^{KBr}: 1740 cm^{-1} (C=O). PMR (in $CDCl_3$) δppm: 0.89 (3H, d6, >CH–C<u>H</u>$_3$), 1.29 (3H, t 7, –CO_2CH_2C<u>H</u>$_3$), 2.48 (3H, s, >N–C<u>H</u>$_3$), 3.22 (1H, m, >C<u>H</u>–CH_3), 3.36 (1H, s, >N–C<u>H</u>$_2CO_2$–), 3.39 (1H, s, >N–C<u>H</u>$_2CO_2$–), 4.22 (2H, quartet 7, –CO_2C<u>H</u>$_2CH_3$), 4.24 (1H, d 8.5, C_6H_5S–C<u>H</u>–CH<), 7.25 (10H, m, aromatic-<u>H</u>). Desulfuration of VII in EtOH by use of Raney Nickel was carried out by usual manner to give rise to 1-phenyl-2-(N-ethoxycarbonylmethyl-N-methyl)aminopropane (II) in 68% of yield, which is coincident in every respect with the authentic sample of II prepared directly from I as mentioned above. Hydrolysis of II by the same method as described before furnished the corresponding acid identical with the aforementioned III.

Methamphetamine–BSA conjugate (VIII) was prepared by conjugation of III to BSA by mixed anhydride method.[3] 10—20 hapten molecules were found to combine with one BSA molecule in VIII by the fluorometric determination in the presence of Marquis reagent[4] (Ex. 400 nm, Em. 465 nm).

Male white rabbits were subcutaneously inoculated with 5 mg of VIII emulsified in an equal volume of complete Freund's adjuvant and saline. A booster injection was given every two weeks. The blood was collected twice 8 and 16 weeks later from the first injection The sera thus obtained were tested for the antibody production by means of the ring test or Ouchterlony method,[5] and the result is shown in Table I. The aforementioned data

Table I. Ring Test and Ouchterlony for Antiserum prepared by using N-Carboxymethylmethamphetamine-BSA Conjugate (VIII)

	Antiserum	Antiserum[a)]
Methamphetamine-BSA(VIII)	+	+
BSA	+	–
Methamphetamine-EA (IX)	+	+
EA[b)]	–	–

a) absorbed with BSA *b*) egg albumin

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suggest the existence of the specific antibody reacted with methamphetamine moiety in the serum.

The radioimmunoassay for methamphetamine using this antibody will be described in the following communication.

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The Radioimmunoassay for Methamphetamine[1)]

The radioimmunoassay for methamphetamine (I) in urine of man has been established by use of the ^{125}I-labelled derivative of N-[3-(*p*-hydroxyphenylacetylamino)propyl]-methamphetamine (V) in sensitivity of 8.0 ng/100 μl.

The antigen binding capacity of the antiserum prepared from N-carboxymethyl-methamphetamine-BSA (II) was determined by a new method of immunoassay using fluorescence labelled N-(3-dansylaminopropyl)methamphetamine (IV) ("fluoroimmunoassay").

The specificity of the antibody was examined by its cross reaction with several methamphetamine analogues.

Keywords——radioimmunoassay; "fluoroimmunoassay"; methamphetamine; N-carboxymethylmethamphetamine-BSA conjugate; N-(3-dansylaminopropyl)methamphetamine; N-[3-(*p*-hydroxyphenylacetylamino)propyl]methamphetamine; ^{125}I-iodination; specificity of antiserum; antihyptonic

Methamphetamine replaced in blood and urine of man is usually determined by gas– and thin–layer chromatography.[2)] These methods, however, involve a number of technical problems in the practical measurement of the compound in biological samples. Since a more rapid, highly specific and much sensitive technique in the determination has obviously been requisite for forensic and clinical purposes, an application of recent development of radioimmunoassay must be invaluable for achievement of the purpose. As far as we are aware, Cheng, *et al.* has reported the radioimmunoassay for amphetamines using ^{3}H-labelled amphetamine.[3)] We now wish to communicate in the present paper a more convenient way of determination of methamphetamine (I) in urine of man by means of the radioimmunoassay

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