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Reaction of Guanidines with α-Diketones. VI.[1] Structures of Fluorescent Products of Biguanides with 9,10-Phenanthraquinone

Shinzo Tanabe and Takeichi Sakaguchi

Faculty of Pharmaceutical Sciences, Chiba University[2]

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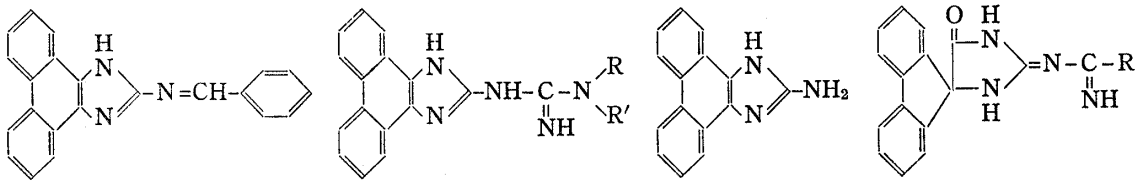
The structures of the fluorescent products produced by the reaction of butylbiguanide, β-phenethylbiguanide, and N,N-dimethylbiguanide with 9,10-phenanthraquinone in the presence of alkali were investigated.

These biguanides gave a free base (IIIb) or ethanol adducts (IVb and Vb) of 2-alkylguanidino-1*H*-phenanthro[9,10-*d*]imidazoles from alkaline solution and their hydrochloride monohydrates (IIIa and IVa) or dihydrochloride (Va) from acidic solution. The structure of these products was determined from their elemental analyses and nuclear magnetic resonance, mass, and high-resolution mass data.

Keywords——fluorescence reaction; biguanides; hypoglycemic agents; 2-alkylguanidino-1*H*-phenanthro[9,10-*d*]imidazole; high-resolution mass spectrometry

Yamada and Itano[3,4] have found the fluorescence reaction with 9,10-phenanthraquinone reagent as a sensitive test for arginine and arginine-containing peptides, and elucidated the structure of the fluorescent product isolated from a mixture of 9,10-phenanthraquinone and arginine to be 2-amino-1*H*-phenanthro[9,10-*d*]imidazole (I). In the previous paper, we reported application of this fluorescence reaction to the fluorometric determination of guanidine.[5] We also isolated 2-benzylideneamino-1*H*-phenanthro[9,10-*d*]imidazole (II) as an intermediate of the fluorescent product (I) from benzylguanidine and discussed the mechanism of this fluorescence reaction.[1] 9,10-Phenanthraquinone also reacted with biguanides to give fluorescence product.[5]

This paper describes the isolation and structural elucidation of the fluorescent products derived from butylbiguanide (Buformin), β-phenethylbiguanide (Phenformin), and N,N-dimethylbiguanide (Methoformin). These biguanides are known as oral hypoglycemic agents.



II

III: R=H, R′=$(CH_2)_3CH_3$

IV: R=H, R′=$(CH_2)_2$-C$_6$H$_5$

V: R=R′=CH_3

I

VI: R=arylamino

Chart 1

1) Part V: S. Tanabe and T. Sakaguchi, *Chem. Pharm. Bull.* (Tokyo), **26**, 337 (**1978**).
2) Location: *1–33, Yayoi-cho, Chiba, 280, Japan.*
3) S. Yamada and H.A. Itano, *Biochim. Biophys. Acta*, **130**, 538 (1966).
4) H.A. Itano and S. Yamada, *Anal. Biochem.*, **48**, 483 (1972).
5) T. Sakaguchi, S. Tanabe, H. Yagi, T. Miyawaki (née Iijima), and A. Saito, *Yakugaku Zasshi*, **97**, 1053 (1977).

Experimental

Materials——The following materials were prepared according to the methods described in the literature: Butylbiguanide hydrochloride,[6] β-phenethylbiguanide hydrochloride,[7] N,N-dimethylbiguanide hydrochloride,[6] 2-amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate[3] (I).

Apparatus——Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Ultraviolet (UV) absorption spectra were measured with Hitachi autorecording spectrophotometer EPS-3T, fluorescence spectra with Hitachi MPF-2A fluorescence spectrophotometer, infrared (IR) spectra with Hitachi IR-215, and nuclear magnetic resonance (NMR) spectra were taken at 100 MHz with JMN 4H-100 spectrometer. The chemical shifts are given in ppm from tetramethylsilane as an internal standard. Abbreviations used: s=singlet, m=multiplet, d-d=double doublet, t=triplet, q=quartet, b=broad. Mass spectra (MS) weretaken with Hitachi RMU-6E spectrometer. High-resolution mass spectral analyses were carried out with JMS-0ISG-2 mass spectrometer (Japan Electron Optics Laboratory Co.).

Isolation of Fluorescent Products from Biguanides and 9,10-Phenanthraquinone——General Procedure: To a suspension of 0.0025 mol of 9,10-phenanthraquinone in 150 ml of EtOH were added, first 0.0025 mol of biguanides dissolved in 11 ml of H_2O, and then 20 ml of 2 M NaOH. The reaction mixture was stirred for 3 hr at room temperature.

i) Reaction Products in Acidic Solution: To the above reaction mixture was added conc. HCl until a white precipitate appeared. The precipitate was collected by filtration and recrystallized from the solvents described in Table I to give IIIa from butylbiguanide, IVa from β-phenethylbiguanide, and Va from N,N-dimethylbiguanide. The yields and physical properties of these compounds are also given in Table I.

ii) Reaction Products in Alkaline Solution: To the above alkaline mixture H_2O was added until a yellow precipitate appeared. The precipitate was collected by filtration and recrystallized from the solvents described in Table I to give IIIb from butylbiguanide, IVb from β-phenethylbiguanide, and Vb from N,N-dimethylbiguanide. The yields and physical properties are also given in Table I.

Table I. Reaction Products of Biguanides with 9,10-Phenanthraquinone

Compound		Mol. wt.[a] M^+ (m/e)	Yield (%)	mp (°C)	Appearance (recryst. solvent)	Analysis (%) Calcd. (Found) C	H	N
IIIa	$C_{20}H_{21}N_5 \cdot HCl \cdot H_2O$	331	43	165—166	Colorless needles (EtOH–H_2O)	62.25 (61.81)	6.27 (6.21)	18.15 (17.84)
IIIb	$C_{20}H_{21}N_5$	331	62	219—220	Pale yellowish plates (EtOH–H_2O)	72.48 (72.27)	6.39 (6.39)	21.13 (21.13)
IVa	$C_{24}H_{21}N_5 \cdot HCl \cdot H_2O$	379	46	211—212	Colorless needles (EtOH)	66.43 (66.33)	5.58 (5.47)	16.14 (16.16)
IVb	$C_{24}H_{21}N_5 \cdot C_2H_5OH$	379	52	128—130	Pale yellowish needles (EtOH–H_2O)	73.38 (73.16)	6.40 (6.57)	16.46 (16.13)
Va	$C_{18}H_{17}N_5 \cdot 2HCl$	303	27	197—198	Colorless needles (EtOH)	57.45 (57.70)	5.09 (5.06)	18.61 (18.39)
Vb	$C_{18}H_{17}N_5 \cdot C_2H_5OH$	303	46	160—161	Pale yellowish needles (EtOH)	68.74 (68.67)	6.63 (6.62)	20.04 (20.02)

a) Molecular weight was measured with mass spectrometer.

Results and Discussion

The elemental analyses and mass spectral data given in Table I indicated that the fluorescent products were formed through the condensation of one equivalent each of biguanides and 9,10-phenanthraquinone with loss of water and oxygen, and these products were also obtained

6) K. Sugino, *Yuki Gosei Kagaku Kyokaishi*, 13, 307 (1955).
7) S.L. Shapiro, V.A. Parrino, and L. Freedman, *J. Am. Chem. Soc.*, 81, 3728 (1959).

as hydrochloride monohydrates (IIIa and IVa) or dihydrochloride (Va) from acidic solution, and as a free base (IIIb) or ethanol adducts (IVb and Vb) from alkaline solution, while the reaction product derived from benzylguanidine was obtained as a Schiff base (II) from alkaline solution and a hydrolyzed product (I) of II from acidic solution.[1)]

Table II. Maxima of Excitation and Emission of Fluorescent Products obtained from Biguanides and 9,10-Phenanthraquinone

Fluorescent Product	Acidic Solution (a)[a)]		Alkaline Solution (b)[a)]	
	Excitation (nm)	Emission (nm)	Excitation (nm)	Emission (nm)
III	260, 300	412	260, 300	440
IV	262, 302	432	262, 302	448
V	272, 304	436	272, 304	444
I[b)]	252, 304	448		

The fluorescent products were dissolved in 95% EtOH and excitation and emission spectra (uncorr.) were measured after 1: 40 dilution with H_2O.
a) See Experimental and Table I.
b) 2-Amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate.

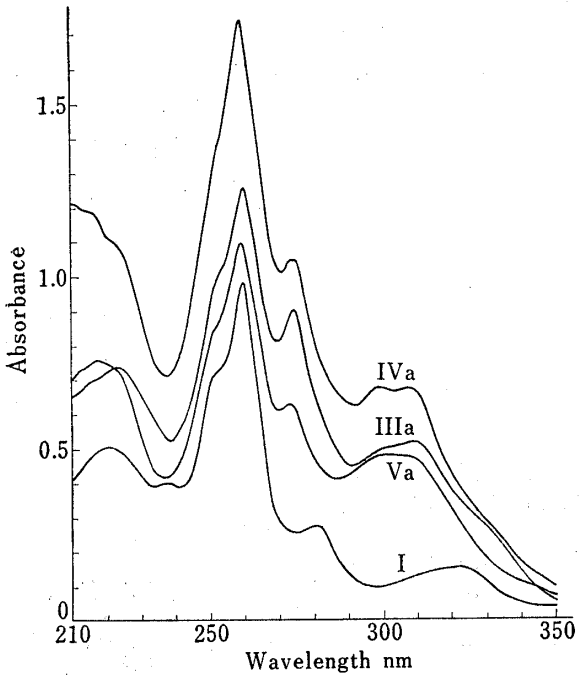


Fig. 1. UV Spectra of Fluorescent Products isolated from Acidic Solution in 95% EtOH
IIIa: 0.025 mM, IVa: 0.044 mM, Va: 0.022 mM, I: 0.016 mM

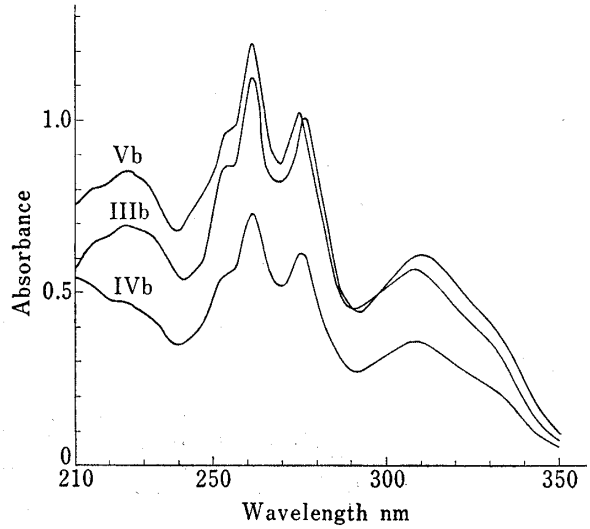
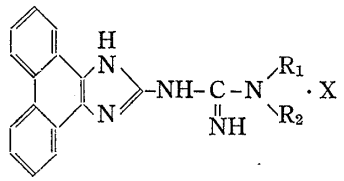


Fig. 2. UV Spectra of Fluorescent Products isolated from Alkaline Solution in 95% EtOH
IIIb: 0.024 mM, IVb: 0.028 mM, Vb: 0.020 mM

The maxima of the excitation and emission spectra, and UV spectra of these fluorescent products are shown in Table II and in Fig. 1 and 2, for comparison with those of 2-amino-1*H*-phenanthro[9,10-*d*]imidazole hydrochloride monohydrate (I). The excitation maximum of each fluorescent product obtained from the acidic solution was identical with that obtained from the alkaline solution, but the emission maximum differed from that obtained from the alkaline solution. On the other hand, the shape of UV spectrum of each fluorescent product obtained from the acidic and alkaline solution was similar to that of I (Fig. 1 and 2). These facts suggest that these fluorescent products are a phenanthroimidazole derivative.

Recently, Furukawa, *et al.*[8] have reported that the base-catalysed condensation of arylbiguanides with 9,10-phenanthraquinone gave N-(1′,5′-dihydro-5′-oxospiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-2′-yl)-N′-arylguanidines (VI) in which the phenanthrene ring underwent 1,2-shift similar to benzilic acid rearrangement. In our preceding paper,[1] it was reported that amidines also reacted with 9,10-phenanthraquinone in alkaline solution to give 2′-(substituted)-spiro[9*H*-fluorene-9,4′-[4*H*]imidazol]-5′(3′*H*)/5′(1′*H*)-ones which involved an intramolecular rearrangement similar to that of VI. From the elemental analyses shown in Table I, however, the fluorescent products from biguanides was not consistent with VI. In fact, the NMR spectra of these fluorescent products showed eight protons due to the phenanthrene ring at 7.56—7.70 (4H, m), 8.32—8.48 (2H, d–d or m), and 8.68—8.88 ppm (2H, d–d), and one proton due to NH of the phenanthroimidazole at 12.02 (IIIb and IVb) and 11.92 ppm (Vb), similar to those of I and II,[1] as shown in Table III. IR spectra of the fluorescent products exhibited an absorption of the guanidine group at 1620—1690 cm^{-1} (IIIa, IVa and Va) or 1615—1630 cm^{-1} (IIIb, IVb and Vb) (Table III).

Table III. NMR and IR Data of Fluorescent Products



	III		IV		V	
	R_1=H, R_2=$(CH_2)_3CH_3$		R_1=H, R_2=$(CH_2)_2$-C$_6$H$_5$		R_1=R_2=CH_3	
	a	b	a	b	a	b
	$HCl \cdot H_2O$		$HCl \cdot H_2O$	C_2H_5OH	2HCl	C_2H_5OH
IR(ν_{max}^{KBr} cm^{-1})						
–NH–C–N< (NH)	1615 1630 1635	1620 1650 1660(sh) 1680	1610 1620 1670	1620(sh) 1650 1660 1680	1610 1630	1620 1640 1662 1690
NMR(δ ppm) in DMSO-d_6						
C–CH_3	0.98(3H, t)	0.98(3H, t)				
C–CH_2	1.54(4H, m)	1.60(4H, m)	2.90(2H, t)	3.00(2H, t)		
C–C_6H_5			7.32(5H, s)	7.32(5H, m)		
N–CH_3					3.04(6H, s)	3.18(6H, s)
N–CH_2	3.32(2H, m)	3.42(2H, m)	3.52(2H, t)	3.68(2H, m)		
Phenanthrene, H	7.60(4H, m) 8.34 (2H, m) 8.74 (2H, d-d)	7.68(4H, m) 8.44 (2H, d-d) 8.80 (2H, d-d)	7.58(4H, m) 8.34 (2H, d-d) 8.74 (2H, d-d)	7.68(4H, m) 8.40 (2H, m) 8.84 (2H, d-d)	7.56(4H, m) 8.32 (2H, m) 8.68 (2H, d-d)	7.70(4H, m) 8.48 (2H, d-d) 8.88 (2H, d-d)
–NH =NH	3.32(m)	3.42(m)	3.28(2H, m) 4.28(1H, m)	3.40(m)	3.28(1H, m) 4.28(1H, m)	5.32(b)
Imidazole, NH	12.02(1H, m)		12.02(1H, m)		11.92(1H, m)	
C_2H_5OH			1.04(3H, t) 3.52(2H, q)		1.08(3H, t) 3.44(2H, q)	

a) Following abbreviations are used: sh: shoulder, s: singlet, d-d: double-doublet, t: triplet, q: quartet, m: multiplet, b: broad.

From these results, the structure of the fluorescent products obtained from biguanides with 9,10-phenanthraquinone was elucidated as 2-alkylguanidino-1*H*-phenanthro[9,10-*d*]imidazoles.

8) M. Furukawa, T. Yoshida, and S. Hayashi, *Chem. Pharm. Bull.* (Tokyo), **23**, 580 (1975).

Mass spectral data of IIIa, IVa, and Va also supported the structures of these products. IIIa, IVa and Va showed common fragment ions at *m/e* 233, 206, 205, 191, 190, 180, 178, 177, 164, 163, 152, and 151, and these ions were quite identical with those of I (M^+: *m/e* 233) whose fragmentation pattern had already been elucidated by Itano and Yamada.[4] This result strongly suggested that the fluorescent products had a similar skeleton to that of phenanthroimidazole. High-resolution mass data of IIIa, IVa, and Va of high mass region above *m/e* 233 (h) are summarized in Table IV. These fragment ions suggested the presence of a guani-

Table IV. High Resolution Data for Fluorescent Products

		IIIa			IVa		
	m/e	Elemental composition (%; relative intensity)	Observed	Calcd.	Elemental composition (%; relative intensity)	Observed	Calcd.
	379				$C_{24}H_{21}N_5$ (61.4)	379.1813	379.1796
	378				$C_{24}H_{20}N_5$ (2.4)	378.1734	378.1718
	363				$C_{24}H_{19}N_4$ (3.5)	363.1555	363.1600
	362				$C_{24}H_{18}N_4$ (11.1)	362.1517	362.1530
	361				$C_{24}H_{17}N_4$ (0.4)	361.1491	361.1453
	331	$C_{20}H_{21}N_5$ (80.0)	331.1833	331.1796			
	330	$C_{20}H_{20}N_5$ (3.0)	330.1754	330.1718			
	316	$C_{19}H_{18}N_5$ (0.5)	316.1486	316.1561			
	315	$C_{19}H_{17}N_5$ (4.6)	315.1497	315.1484			
	314	$C_{20}H_{18}N_4$ (28.3)	314.1500	314.1530			
	289	$C_{17}H_{15}N_5$ (0.9)	289.1310	289.1326	(4.7)	289.1253	289.1326
a	288	$C_{17}H_{14}N_5$ (3.2)	288.1217	288.1248	(27.4)	288.1237	288.1248
b	286	$C_{18}H_{14}N_4$ (0.5)	286.1166	286.1217	(0.6)	286.1206	286.1218
c	271	$C_{17}H_{11}N_4$ (0.4)	271.1030	271.0984	(1.6)	271.0983	271.0983
d	260	$C_{16}H_{12}N_4$ (5.4)	260.1022	260.1060	(6.7)	260.0989	260.1061
e	259	$C_{16}H_{11}N_4$ (34.1)	259.0972	259.0982	(33.5)	259.0955	259.0982
f	258	$C_{16}H_{10}N_4$ (100.0)	258.0916	258.0905	(61.7)	258.0902	258.0904
g	257	$C_{16}H_9N_4$ (5.0)	257.0779	257.0826	(8.0)	257.0758	257.0827
h	233	$C_{15}H_{11}N_3$ (62.6)	233.0926	233.0951	(100.0)	233.0909	233.0952

		Va		
	m/e	Elemental composition (%; relative intensity)	Observed	Calcd.
	303	$C_{18}H_{17}N_5$ (75.8)	303.1467	303.1482
	302	$C_{18}H_{16}N_5$ (3.6)	302.1374	302.1404
a	288	(0.6)	288.1295	288.1249
b	286	(0.5)	286.1246	286.1218
c	271	(2.5)	271.0928	271.0982
d	260	(6.4)	260.1062	260.1061
e	259	(33.4)	259.0950	259.0982
f	258	(100.0)	258.0903	258.0904
g	257	(3.7)	257.0778	257.0826
h	233	(30.5)	233.0939	233.0952

dino group. Namely, the fragment ions corresponding to the loss of $\cdot NH_2$, NH_3, or $\cdot NH_4$ from the molecular ion were appeared at *m/e* 314 (IIIa: M^+-NH_3), 363 (IVa: $M^+-\cdot NH_2$), 362 (IVa: M^+-NH_3), 361 (IVa: $M^+-\cdot NH_4$), and 286 (Va: b, M^+-NH_3). These ions were the characteristic peaks of guanidines as reported by Beynon, *et al.*[9] Further, mass spectra of

9) J.H. Beynon, J.A. Hopkinson, and A.E. Williams, *Org. Mass Spectrum.*, **1**, 169 (1968).

the three fluorescent products showed the common fragment ion peaks at *m/e* 288 (a) to 233 (h). In these ions, a was formed by the loss of $\cdot C_3H_7$ (IIIa), $\cdot C_7H_7$ (IVa), and $\cdot CH_3$ (Va) from the molecular ions and c (*m/e* 271) was formed by the loss of NH_3 from the ion a. The ion b (*m/e* 286) was probably formed from the ions at *m/e* 314 of IIIa and *m/e* 362 of IVa by the loss of C_2H_4 and C_6H_4, respectively. In the case of Va, the ion b was formed directly from the molecular ion by the loss of NH_3. IIIa, IVa, and Va also formed a common peak at *m/e* 260 (d). The fragment ion at *m/e* 260 was produced from the ion at *m/e* 289 (IIIa: $M^+-C_3H_6$, IVa: $M^+-C_7H_6$) with accompanying migration of the methyl group to the imino-nitrogen as observed in methyl- and N,N-dimethylguanidines.[10] In the case of Va, the fragment ion at *m/e* 260 was produced by the loss of C_2H_5N from the molecular ion and transfer of a hydrogen as observed in N,N,N′,N′-tetramethylguanidine.[9] The ion d (*m/e* 260) further decayed to the ion at *m/e* 233 (h) by the loss of HCN, whose elementary composition was the same as that of I. This ion also lost a hydrogen to give the ion at *m/e* 259 (e). The ion at *m/e* 258 (f), which was the base peak in IIIa and IVa, and the second intense peak in Va, was probably formed from ion e by loss of $\cdot H$, and this ion peak would be also formed from molecular ions by loss of a RR′NH group.[9] The ion f then lost $\cdot H$ to form the ion at *m/e* 257 (g). Possible structures of the fragment ions and the fragmentation paths from d to h are illustrated in Chart 1.

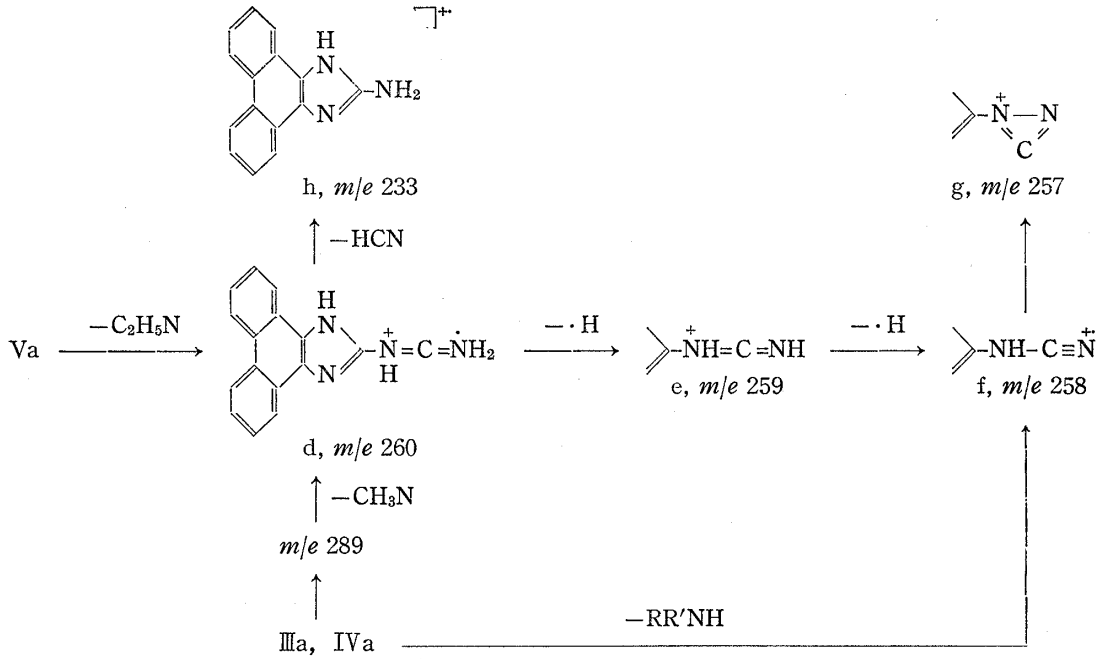


Chart 2. Possible Structure of the Fragmentations of d to h

It was further evident from the foregoing mass assigments that the structure of the fluorescent products of biguanides with 9,10-phenanthraquinone was 2-alkylguanidino-1*H*-phenanthro[9,10-*d*]imidazoles.

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10) A.G. Loudon, A. Maccol, and K.S. Webb, *Adv. Mass Spectrom.*, **4**, 223 (1968).