CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 16 No. 8 **August 1968**

[Chem. Pharm. Bull. 16(8)1417—1432(1968)] *UDC 615.244.011: 547.466.2.07*

Studies on Hepatic Agents. I. Synthesis of Aminoacyl (and Hydroxyacyl) Aminoacetonitriles

SEIGO SUZUE and TUTOMU IRIKURA

Kyorin Chemical Laboratory[1]

(Received May 20, 1967)

For the purpose of elucidating the structural relationship between lathyrism and inhibition of necrosis induced by CCl_4 in the liver of the rat, many compounds related to aminoacetonitrile were synthesized. N–Aminoacylaminoacetonitriles (VIII, XIV) were synthesized from phthaloylaminoacylaminoacetonitriles. VIII were converted to 2–hydroxyimino–5–oxopiperazine derivatives (XV) and 5–oxo–2–thio–piperazines (XX). Preparations of N–(N–acylaminoacyl)aminoacetonitriles were also described. Further, N–α–hydroxyacylaminoacetonitriles were prepared from chloralides of α–hydroxyacids with aminoacetonitrile. In addition, XV (R=H) was converted to 2–acetamino–5–acetoxypyrazine by treatment with acetic anhydride.

It has been known that aminoacetonitrile (abbreviated hereafter to AAN), having strong lathyrogenic action, inhibits necrosis induced by carbontetrachloride in the liver of the rat.[2] But the lathyrism is too strong to use as a remedy. Then it was considered to be necessary to improve the chemical structure to reduce the toxicity without loss of its efficiency.

For the purpose of elucidating the structural relationship between lathyrism and inhibition of necrosis in the liver, a number of compounds related to AAN were synthesized. Initially, as AAN analogues, α and β–aminopropionitrile, N,N–dialkyl–AANs,ω–aminoalkylnitriles, α–aminoalkylnitriles, α–cyanopyrrolidine, glycinamide, and methylene–AAN were synthesized and tested their antinecrotic action, but they showed almost no activity. But as the results of investigation of several kinds of N–acyl–AANs, it was found that N–aminoacyl–AANs (VIII and XIV) and some of N–(N–acylaminoacyl)–AANs (XXV) had lower toxicity and lathyrism than those of AAN and antinecrotic action in the liver of the rat.

Synthesis of aminoacyl–AAN analogues could not be found in previous reports, but 3–(N–γ,L–glutamoyl) aminopropionitrile being in a sweet pea as a lathyrogen was synthesized.[3]

The syntheses were carried out as shown in Chart 1. N–Chloroacetyl–AAN (I) prepared from chloroacetylchloride and AAN was treated with 28% aqueous ammonia according to the usual method of peptide syntheses, afforded the products which were proved by paperchromatography[4] to be a mixture of N–glycyl–AAN (II: R=R′=H), *Rf* 0.44, and N–glycylglycinamide

---

[1] Location: *1–3, Ukima, Kita–ku, Tokyo.*
[2] L. Fium, *Nature*, **197**, 397 (1963).
[3] E.D. Schilling and F. M. Strong, *J. Am. Chem. Soc.*, **77**, 2843 (1955).
[4] Toyo filter paper No. 50, BuOH—AcOH—H_2O (4:1:2), Ninhydrin test.

(III: R=R′=H), *Rf* 0.27. And the separation of the mixture by fractional crystallization was unsuccessful.

Treating with excess aqueous dimethylamine or piperidine instead of ammonia, I afforded the corresponding amide (III). But with an equimolar amount of the amine in the presence of neutralizing agent in aqueous solution, I gave the corresponding II without hydrolysis of the nitrile. And also with anhydrous dimethylamine or diethylamine I was converted to II without damage to the nitrile.

N–Glycylaminoacetonitrile and its analogues (VIII) were obtained as the acetic acid salts by dephthaloylation[5] of N–(N–phthaloylaminoacyl)–AANs (VII) which were prepared from N–*α*–haloacyl–AANs (VI) and potassium phthalimide, or phthaloylaminoacid chlorides (IV) and AAN. But the yields of the dephthaloylation were poor and inconstant, because the nitrile being sensitive to hydrazine or amine probably gave rise to some side reaction during the reaction and the treatment.

Optically active L–leucyl–AAN (VIII: $R=CH_2CH(CH_3)_2$) was prepared from L–phthaloylleucine obtained by the method of Nefkins, *et al.*, [6] *via* IV to VII. The infrared spectra of VIII derivatives in KBr–Tablet did not show clearly the absorption bands of the nitriles. Then it was considered that *e.g.*, VIII (R=H) might be the isomer, 2–imino–5–oxopiperazine acetate, which effected by condensation of the *α*–amino group and the endo nitrile. But this was denied by following examination. The product prepared from VIII (R=H) and benzyloxycarbonylchloride was identical with N–(N–benzyloxycarbonylglycyl)–AAN (X) prepared from N–benzyloxycarbonylglycylchloride (IX) and AAN.

When X was treated with hydrogenbromide in acetic acid, N–glycylglycinamide hydrobromide (XI) was obtained with almost quantative yield. Attempts to convert X to VIII (R=H) by catalytic reduction over paradium–black were unsuccessful, because of the reduction of the nitrile. And VIII (R=H) was not recognized by the detection of paperchromato-

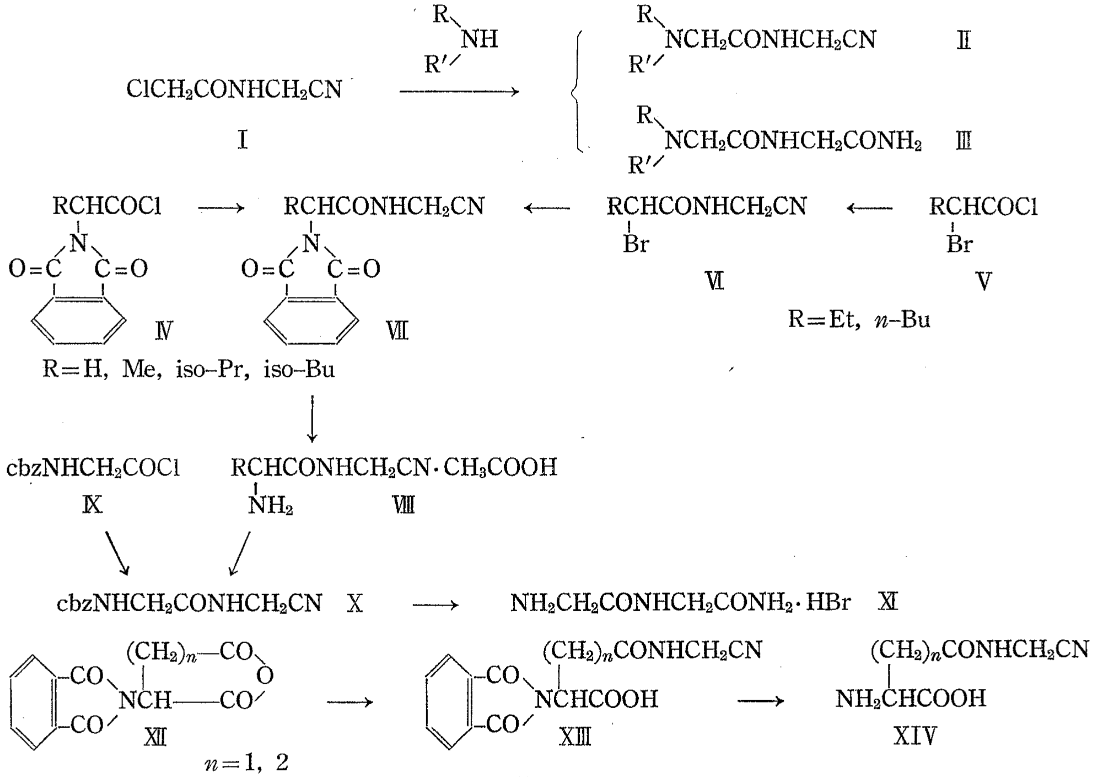


Chart 1

---

5) J.C. Sheehan and V. S. Frank, *J. Am. Chem. Soc.*, **71**, 1856 (1949).

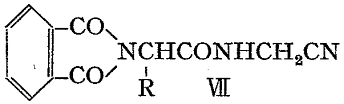
6) G.H.L. Nefkins, G.I. Tesser, and R.T.F. Nivard, *Rec. Trav. Chim.*, **79**, 688 (1960) [*C. A.*, **55**, 1461*h* (1961)].

TABLE I. $^{R}_{R'}>NCH_2CONHCH_2CN$ II

Compd. No.	R	R_1	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. H	Found C	Found H	Found N	Recryst. solvent[a)]	Appearance[b)]	mp (°C)	Yield (%)
1	CH_3-	CH_3-	$C_6H_{11}ON_3$	51.04	7.85	29.77	50.78	7.85	30.00	—	n	65—68	82[c)]
	picrate		$C_{12}H_{14}O_8N_6$	38.92	3.81	22.70	39.22	4.10	22.98	H	yellow n	176—177	
2	CH_3CH_2-	CH_3CH_2-	$C_8H_{15}ON_3$	56.78	8.94	24.83	55.97	8.98	24.32	—	n	34—37	70
	picrate		$C_{14}H_{18}O_8N_6$	42.21	4.55	21.10	42.61	4.92	21.43	H	yellow n	178—179	
3	C_6H_5-	H-	$C_{10}H_{11}ON_3$	63.47	5.86	22.21	63.49	5.95	22.46	B	n	137—140	96
4	o-HOOC·C_6H_4-	H-	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	56.82	4.79	18.03	E	n	210	78
5	m-HOOC·C_6H_4-	H-	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	56.16	5.01	17.79	H	n	173—174	56
6	p-HOOC·C_6H_4-	H-	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	56.55	4.59	17.85	H	n	215—217	42
7	o-HOOC·C_6H_4-	CH_3CO-	$C_{13}H_{13}O_4N_3$	56.72	4.76	15.27	56.77	5.22	15.36	H	n	174—176	39
8	$<^{CH_2CH_2}_{CH_2CH_2}>$		$C_{12}H_{18}O_2N_6$	51.78	6.52	30.20	51.06	6.27	30.89	H+E	n	231—233	71
9	 $-CH_2\underset{COOH}{CH}-$	H-	$C_{13}H_{15}O_3N_3$	59.76	5.79	16.08	59.31	5.87	16.18	H+E	n	206—209	48

[a)] A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
[b)] Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
[c)] prepared from VIII (R=H)

TABLE II.



Ⅶ

Compd. No.	R	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a)]	Appearance[b)]	mp (°C)	Yield (%)
10	H-	$C_{12}H_9O_3N_3$	—	—	−17.28	—	—	17.56	N	n	245—246	88
11	CH_3	$C_{13}H_{11}O_3N_3$	60.69	4.31	16.34	60.64	4.35	16.69	E	n	175—176	91
12	$(CH_3)_2CH-$	$C_{15}H_{15}O_3N_3$	63.15	5.30	14.73	62.98	5.21	15.19	E	n	170—172	92
13	$(CH_3)_2CHCH_2-$	$C_{16}H_{17}O_3N_3$	64.20	5.72	14.04	64.20	5.61	14.55	60% E	n	114—118	75

[a)] A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
[b)] Small n shows colorless needles; l, colorless leaflet; p, colorless plates.

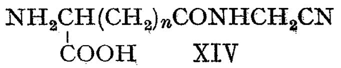
TABLE III.

$NH_2\underset{R}{C}HCONHCH_2CN \cdot CH_3COOH$ VIII

Compd. No.	R	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a)]	Appear-ance[b)]	mp (°C)	Yield (%)
14	H–	$C_6H_{11}O_3N_3$	41.61	6.40	24.27	41.56	6.46	24.18	95% E	n	139—140	67
15	CH_3–	$C_7H_{13}O_3N_3$	44.91	7.00	22.45	44.91	6.90	22.87	N	n	105—107	40
16	$(CH_3)_2CH$–	$C_9H_{17}O_3N_3$	50.22	7.96	19.52	50.00	7.79	19.95	E	n	156—157	53
17	$(CH_3)_2CHCH_2$–	$C_{10}H_{19}O_3N_3$	52.38	8.35	18.33	52.07	8.08	19.12	N	n	111—113	72
18	CH_3CH_2–	$C_8H_{15}O_3N_3$	47.75	7.51	20.88	47.70	7.46	21.24	N	n	147—149	53[c)]
19	$CH_3(CH_2)_3$–	$C_{10}H_{19}O_3N_3$	52.38	8.35	18.33	52.38	8.37	18.55	N	n	126—127	37[c)]

a) A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
b) Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
c) calcd. from VI

TABLE IV.



$NH_2\underset{COOH}{C}H(CH_2)_nCONHCH_2CN$ XIV

Compd. No.	n	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a)]	Appear-ance[b)]	mp (°C)	Yield[c)] (%)
20	1	$C_6H_9O_3N_3$	42.10	5.30	24.55	41.80	5.11	24.32	H	n	213—218	67
21	2	$C_7H_{11}O_3N_3$	45.40	5.99	22.69	45.73	6.11	23.08	H	n	216—218	53

a) A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
b) Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
c) calcd. from XIV

graphy[7] of the reduction mixture. However by catalytic reduction over paradiumblack in the presence of formaline, VIII (R=H) was converted to II ($R=R'=CH_3$) without reduction of the nitrile.

On the other hand, aspartyl and glutamyl derivatives (XIV) were synthesized by applying the method reported by King, *et al.*[8] from (XII) *via* (XIII). Its constitution as a ω–amide was confirmed by the Van Slyke ninhydrin determination,[9] the liberation of carbon dioxide being equivalent to one free α–amino acid residue.

By treating VIII derivatives with hydroxylamine, the corresponding amidoximes were not obtained but cyclic amidoximes (XV) were obtained as shown in Chart 2. Their formulations were assigned but the following facts. There was observed the evolution of ammonia during the reaction and the product showed blue colour with a ferric chloride reagent. Further, the compound (XV: R=H) was converted to 2,5–dioxopiperazine (XVI) by treating with sodium nitrite in aqueous hydrogenchloride. The infrared spectra of XV derivatives showed no absorption bands in the vicinity of 1550 cm^{-1} (amide II), which presented in those of VIII derivatives, and that meaned the formation of lactam system.

N–(N–*n*–Propylglycyl)–AAN (II: R=H, $R'=CH_2CH_2CH_3$) was also converted to the cyclic amidoxime (XVII), while N–(N–phenylglycyl)–AAN (II: R=H, $R'=C_6H_5$) gave the corresponding amidoxime (XVIII) and cyclization did not occur even by refluxing in water. The action of phenylisocyanate to XV (R=H) afforded the O–acyl product (XIX), though some amidoxime have been reported to yield N–acylated compound.[10] The infrared spectrum of XIX revealed the absence of the broad hydroxy peak at 2700—3000 cm^{-1} and the presence of –COO– at 1720 cm^{-1}. Further VIII (R=H and R=iso–Bu) were converted to cyclic thioamides, 2–oxo–5–thiopiperazine derivatives (XX), by treating with hydrogensulfite in

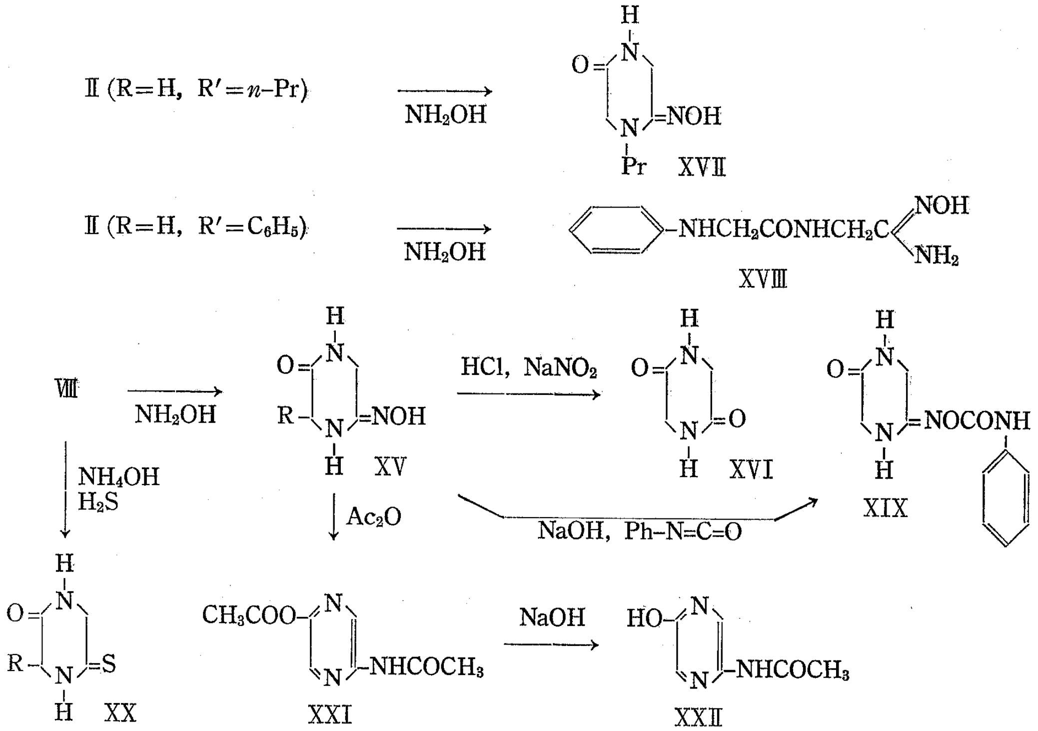


Chart 2

---

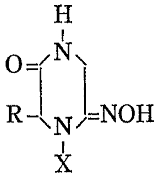
[7] It showed four spots by ninhydrin test, *Rf* 0.38, 0.25, 0.21, and 0.10 (Toyo filter paper No. 50, BuOH—H_2O—AcOH (4:2:1)).

[8] F.E. King and D.A.A. Kidd, *J. Chem. Soc.*, **1951**, 2976; **1949**, 3315.

[9] D.D. Van Slyke, D. A. MacFadyen, and P. Hamilton, *J. Biol. Chem.*, **141**, 671 (1941).

[10] F. Eloy and R. Lenaers, *Chem. Rev.*, **62**, 155 (1962).

TABLE V.



XV and XVII

Compd. No.	R	X	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a)]	Appearance[b)]	mp (°C)	Yield (%)
99	H	H	$C_4H_7O_2N_3$	37.21	5.46	32.55	37.07	5.35	32.64	H	l	198—199 d[c)]	58
100	$(CH_3)_2CH-$	H	$C_7H_{13}O_2N_3$	49.11	7.65	24.55	49.17	7.53	24.82	E or H	l	184—188 d	60
101	$(CH_3)_2CHCH_2-$	H	$C_8H_{15}O_2N_3$	51.87	8.16	22.69	51.82	7.94	22.52	E	l	164—166 d	66
102	$CH_3(CH_2)_3-$	H	$C_8H_{15}O_2N_3$	51.87	8.16	22.69	51.99	7.82	22.64	H	l	165—166 d	77
103	H	$CH_3(CH_2)_2-$	$C_7H_{13}O_2N_3$	49.11	7.65	24.55	49.22	7.48	24.71	H	p	195—196 d	64

a) A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
b) Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
c) d=decompd.

aqueous ammonia. The infrared spectra of XX in KBr–Tablet showed absorption of carbonyl at 1650 cm^{-1} and thiocarbonyl at 1580 and 1160 cm^{-1}.

When XV (R=H) was refluxed with acetic anhydride to prepare the O–acetyl derivative, unexpected product, $C_8H_9O_3N_3$, mp 154—156°, was obtained. The ultraviolet spectrum of this substance in ethanol exhibited maxima at 239 mμ (ε 14800) and 303 mμ (ε 7800), which suggested the formation of conjugated system since VIII showed no maxima at the region 220—360 mμ. Its infrared spectrum in KBr–Tablet had absorption bands at 3200 (NH), 3000, 1750 (-COO-), 1650 (-CON-), 1590 (-C=C-), and 1540 (amide II) cm^{-1}.

The nuclear magnetic resonance spectrum of the product in deuteriochloroform contained the signals for each doublet one proton at 0.75 τ and 1.90 τ with the coupling constant of 1.6 cps and for each singlet three protons at 7.65 τ and 7.75 τ. The former two signals could be assigned to the protons on the pyrazine ring and the later two signals were appearently due to the protons on the acetoxy and acetamido groups, together with the absorption bands at 1750 cm^{-1} and 1650 cm^{-1} in the infrared spectrum. The above data confirmed that the product

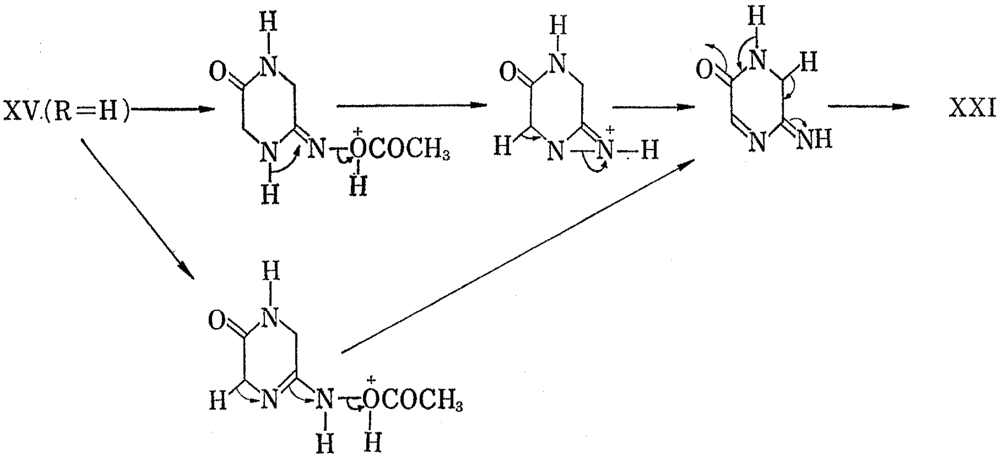


Chart 3

was 2-acetamino-5-acetoxypyrazine. The above reaction is closely related to the Semmler-Wolff aromatization.[11] The mechanism of the reaction is seemed to be similar to that of the Semmler-Wolff aromatization and two ways are postulated on the basis of the reports by Conley[12] and Bhatt[13] as illustrated in Chart 3. Further work on this reaction is in progress. From a practical viewpoint, the reaction will provide a useful method for the synthesis of various 2-aminopyrazine derivatives.

In the meantime, some of the acyl derivatives (XXV) of VIII proved to be particularly interesting and a number of the derivatives (XXV) were prepared in order to better understand the structural requirements for antinecrotic activity and lathyrogenic action. They were prepared by treatment of VIII with acid chloride or acid anhydride (method a and method b) and otherwise from N-acylamino acid (XXIII) and AAN by the action of N,N-dicyclohexylcarbodiimide (abbreviated hereafter to DCC),[14] or oxazolone derivatives (XXIV)[15] and AAN (method c and method d) as shown in Chart 4. Generally, method c was the most brief procedure but purity of the product was so wrong that repeated recrystallizations were necessary, consequently the yields were poor as shown in Table VI–VIII. The compounds summarized in Table VII and VIII were synthesized for the purpose to investigate the relationship between the position of the amino group and the biological activities.

It was also desired to prepare comperatively simple N-acyl-AANs (XXVI) which would be also likely to have the biological activities. They were obtained from AAN by treating with acid chloride, acid anhydride, and acid with DCC (method e, f, and g respectively) as shown in chart 4 and summarized in Table IX. Attemps to prepare malonic monoamide from malonic acid and AAN by the method g were unsuccessful and the product was always the diamide (Compound No. 87) and that might suggest the formation of carbon suboxide at first stage from malonic acid and DCC.

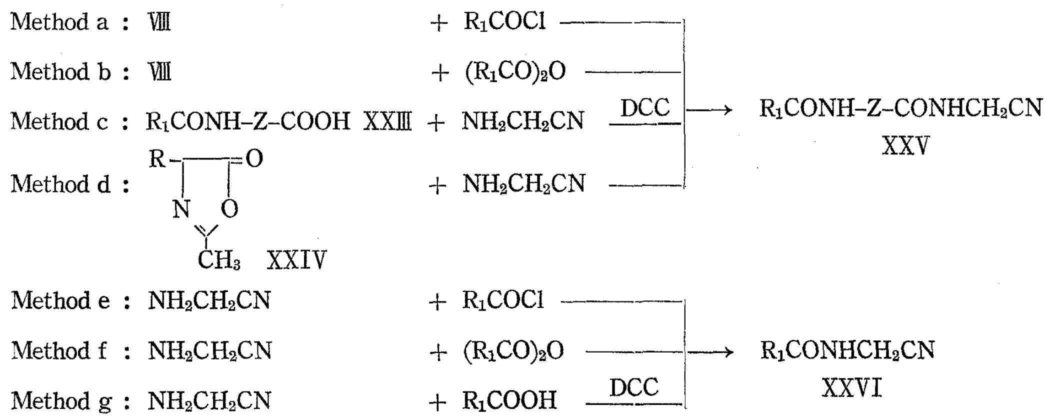


Chart 4

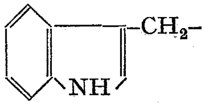
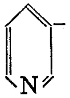
Next, α-hydroxyacyl-AANs were synthesized for the purpose to investigate the variation of the biological activities by the convertion of the amino group to the hydroxy one, as it was considered that the lathyrism of VIII might be due to the amino group.

Davies[16] reported that 1,3-dioxolane-2,4-dione (XXVIII) prepared from glycolic acid (XXVII) and phosgen, reacted with aniline to give N-glycoloylaniline. According to this method, N-glycoloyl-AAN (XXIX) was obtained as shown in Chart 5. But the synthesis

---

11) F.W. Semmler, *Chem. Ber.*, **25**, 3352 (1892); E. C. Horning, V. L. Stvomberg, and H. A. Lloyd, *J. Am. Chem. Soc.*, **74**, 5153 (1952).
12) R.T. Conley, *Experientia*, **18**, 497 (1962).
13) M.W. Bhatt, *Experientia*, **13**, 70 (1957).
14) N.F. Albertson, *Org. Reaction*, **12**, 205 (1962).
15) H.E. Carter, *Org. Reaction*, **3**, 216 (1946).
16) W.H. Davies, *J. Chem. Soc.*, **1951**, 1357.

Table Ⅵ. $R_1CONH\underset{R}{\underset{|}{CH}}CONHCH_2CN$ XXV

Compd. No.	R (R_1=CH_3)	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a]	Appearance[b]	mp (°C)	Yield (%)	Method[c]
22		$C_{15}H_{16}O_2N_4$	63.36	5.67	19.71	63.12	5.47	20.21	N	l	196—197	68	c
23	NH_2COCH_2–	$C_8H_{12}O_3N_4$	45.28	5.70	26.40	45.13	5.67	26.61	90% E	n	195—196	41	c
24	C_6H_5–	$C_{12}H_{13}O_3N_3$	62.32	5.67	18.17	62.20	5.54	18.02	N	n	192—193	37	c
25	$C_6H_5\cdot CH_2$–	$C_{13}H_{15}O_2N_3$	63.66	6.16	17.13	63.61	5.94	17.24	H+E	n	188—189	66	d
26	*p*–AcO·$C_6H_4\cdot CH_2$–	$C_{15}H_{17}O_4N_3$	59.39	5.65	13.86	59.39	5.51	13.52	H	n	179—180	76	d
	R_1 (R=$CH_3S(CH_2)_2$)												
27	CH_3–	$C_9H_{15}O_2N_3S$	47.15	6.60	18.33	47.26	6.66	17.67	P	n	109—111	17	c
28	$(CH_3)_2CHCH_2$–	$C_{12}H_{21}O_2N_3S$	53.12	7.80	15.49	53.16	7.92	15.19	H	n	103—105	23	c
29		$C_{13}H_{16}O_2N_4S$	53.42	5.52	19.17	53.38	5.49	19.37	E	n	140—142	63	c
	R_1 (R=H)												
30	CH_3CH_2–	$C_7H_{11}O_2N_3$	49.69	6.55	24.84	49.62	6.67	24.92	E	n	159—160	75	b
31	$CH_3(CH_2)_2$–	$C_8H_{13}O_2N_3$	52.44	7.15	22.94	51.94	7.13	23.21	E	n	129—131	74	b
32	$(CH_3)_2CH$–	$C_8H_{13}O_2N_3$	52.44	7.15	22.94	52.40	6.82	22.75	A	n	102—104	79	a
33	$CH_3(CH_2)_4$–	$C_{10}H_{11}O_2N_3$	56.85	8.11	19.89	57.56	8.15	20.10	A	l	142—143	81	a
34	$CH_3(CH_2)_5$–	$C_{11}H_{19}O_2N_3$	58.64	8.50	18.65	58.86	8.31	19.00	80% E	l	141—142	60	a
35	CH_2=CH–	$C_7H_9O_2N_3$	50.29	5.43	25.14	50.43	5.14	24.87	H	n	160—161	51	a
36	*trans*–CH_3CH=CH–	$C_8H_{11}O_2N_3$	53.03	6.12	23.19	52.62	5.58	23.06	N	n	169—170	64	b
37	$CH_3(CH_2)_6$–	$C_{12}H_{21}O_2N_3$	60.22	8.85	17.56	60.19	9.00	17.71	E	l	146—147	51	a
38	C_6H_5–	$C_{11}H_{11}O_2N_3$	60.82	5.10	19.35	60.46	4.86	19.53	20% E	n	186—188	88	a
39	*o*–HO·C_6H_4–	$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	56.89	5.01	17.70	E	n	190—191	82	a
40	*p*–$CH_3.C_6H_4$–	$C_{12}H_{13}O_2N_3$	62.32	5.67	18.17	62.22	5.63	18.52	E	n	192—193	90	a
41	*p*–$CH_3O\cdot C_6H_4$–	$C_{12}H_{13}O_3N_3$	58.29	5.30	17.00	58.31	5.36	16.96	E+H	p	166—167	86	a
42	3,4,5–$(CH_3O)_3C_6H_2$–	$C_{14}H_{17}O_5N_3$	54.72	5.58	13.68	54.43	5.70	13.64	E	p	192—193	86	a

43	p-$NO_2.C_6H_4$-	$C_{11}H_{10}O_4N_4$	50.38	3.84	21.37	50.70	4.33	21.13	E	yellow n	193—194	83	a
44	m-$NO_2 \cdot C_6H_4$-	$C_{11}H_{10}O_4N_4$	50.38	3.84	21.37	50.64	4.11	21.60	E	n	180—181	87	a
45	p-$NH_2.C_6H_4$-	$C_{11}H_{12}O_2N_4$	56.89	5.21	24.13	56.68	5.46	24.44	H	n	196—197	97	h
46	m-$NH_2.C_6H_4$-	$C_{11}H_{12}O_2N_4$	56.89	5.21	24.13	56.50	5.21	24.24	H	n	152—153	88	h
47	p-$CH_3CONH \cdot C_6H_4$-	$C_{13}H_{14}O_3N_4$	56.93	5.15	20.43	55.49	5.01	20.02	E	n	237—239	69	d)
48	$CH_3CONHCH_2$-	$C_8H_{12}O_3N_4$	45.28	5.70	26.40	45.70	5.98	26.52	dioxane	n	238—239	68	c
49	RCO-=-p-$CH_3C_6H_4SO_2$-	$C_{11}H_{13}O_3N_3S$	49.43	4.90	15.73	49.47	5.00	16.00	E	n	168—169	89	a
	R_1 (R=CH_3)												
50	CH_3-	$C_7H_{11}O_2N_3$	49.69	6.55	24.84	49.40	6.62	25.19	P	n	149—150	80	b
51	$(CH_3)_2CH$-	$C_9H_{15}O_2N_3$	54.80	7.67	21.37	54.82	7.73	21.16	E	n	165—166	43	b
52	C_6H_5-	$C_{12}H_{13}O_2N_3$	62.32	5.67	18.17	62.24	5.37	18.61	H	n	168—169	79	a
53	$C_6H_5CH_2O$-	$C_{13}H_{15}O_3N_3$	59.76	5.79	16.08	59.78	5.40	16.72	H	n	129—130	83	a
	R_1 (R=$(CH_3)_2CH$)												
54	CH_3-	$C_9H_{15}O_2N_3$	54.80	7.67	21.31	54.69	7.65	21.36	H	n	187—188	69	b
55	CH_3CH_2-	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.93	8.08	20.10	E	n	184—185	78	b
56	$CH_3(CH_2)_2$-	$C_{11}H_{19}O_2N_3$	58.64	8.50	18.65	58.77	8.54	18.78	30% E	n	172—173	67	b
57	$(CH_3)_2CH$-	$C_{11}H_{19}O_2N_3$	58.64	8.50	18.65	58.70	8.39	18.68	E	n	192—193	44	b
58	$CH_3(CH_2)_4$-	$C_{13}H_{23}O_2N_3$	61.63	9.15	16.59	61.52	9.02	16.94	E	n	167—168	37	a
59	$CH_3(CH_2)_5$-	$C_{14}H_{25}O_2N_3$	62.89	9.43	15.72	62.12	9.19	15.75	E	n	167—168	57	a
60	$CH_3(CH_2)_6$-	$C_{15}H_{27}O_2N_3$	64.02	9.67	14.93	64.01	9.55	14.81	60% E	l	148—149	57	a
61	C_6H_5-	$C_{14}H_{17}O_2N_3$	64.84	6.61	16.21	64.68	6.41	16.54	E	n	225—227	79	a
62	p-$NO_2 \cdot C_6H_4$-	$C_{14}H_{16}O_4N_4$	55.25	5.30	18.41	55.31	5.30	18.37	M	yellow n	247—248	76	a
63	p-$NH_2 \cdot C_6H_4$-	$C_{14}H_{18}O_2N_4$	61.29	6.61	20.43	60.91	6.48	20.34	50% E	n	220—221	82	h
	R_1 (R=$(CH_3)_2CHCH_2$)												
64	CH_3-	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.70	8.02	20.01	N	n	144—145	81	b
65	CH_3CH_2-	$C_{11}H_{19}O_2N_3$	58.64	8.50	18.65	58.83	8.74	19.00	H	n	127—128	89	b
66	$(CH_3)_2CH$-	$C_{12}H_{21}O_2N_3$	60.22	8.85	17.56	60.17	8.58	17.60	H	n	134—135	57	b
67	C_6H_5-	$C_{15}H_{19}O_2N_3$	65.91	7.01	15.37	66.38	6.54	15.49	60% E	n	158—159	79	a
68	p-$NO_2 \cdot C_6H_4$-	$C_{15}H_{18}O_4N_4$	56.59	5.70	17.60	56.51	5.88	17.11	60% E	yellow n	164—165	88	a
69	p-$NH_2 \cdot C_6H_4$-	$C_{15}H_{20}O_2N_4$	62.48	6.99	19.43	62.32	7.10	19.24	E	n	194—195	91	h

---

a) A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.

b) Small n shows colorless needles; l, colorless leaflet; p, colorless plates.

c) Small a, b, c, d, e, f, and g show the method a, b, c, d, e, f, and g in each compd., respectively, as described in the experimental. Small h show the compd. was prepd. from the corresponding nitro compd. by catalytic reduction over Pd-black.

d) This compd. was prepared from the compd. no. 45 with Ac_2O.

Table VII. $CH_3CONH(CH_2)_nCONHCH_2CN$ XXV

Compd. No.	n	Formula	Analysis (%) Calcd.			Found			Recryst. solvent[a]	Appearance[b]	mp (°C)	Yield (%)	Method[c]
			C	H	N	C	H	N					
70	1	$C_6H_9O_2N_3$	46.44	5.85	27.08	46.41	7.83	27.35	E	l	157—159	87	b
71	2	$C_7H_{11}O_2N_3$	49.69	6.55	24.85	49.53	6.31	24.80	E	n	143—144	24	c
72	3	$C_8H_{13}O_2N_3$	52.44	7.15	22.94	52.17	6.88	22.69	A	l	89	19	c
73	4	$C_9H_{15}O_2N_3$	54.80	7.67	21.31	54.81	7.71	21.54	A	l	101—102	11	c
74	5	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.20	7.93	19.85	A	p	103—104	41	c

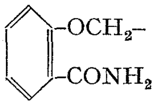
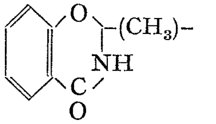
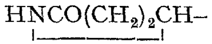
[a] A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
[b] Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
[c] Small a, b, c, d, e, f, and g show the method a, b, c, d, e, f, and g in each compd., respectively, as described in the experimental. Small h show the compd. was prepd. from the corresponding nitro compd. by catalytic reduction over Pd-black.

Table VIII. $CH_3(CH_2)_4CONHCH_2CN$
$NHCOCH_3$ XXV

Compd. No.	Position of AcNH–	Formula	Analysis (%) Calcd.			Found			Recryst. solvent[a]	Appearance[b]	mp (°C)	Yield (%)	Method[c]
			C	H	N	C	H	N					
75	2	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.79	7.68	19.35	A	n	107—108	80	b
76	3	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.68	7.68	19.66	A	n	146—147	23	c
77	4	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	57.09	8.28	20.04	A	n	147—148	15	c
78	5	$C_{10}H_{17}O_2N_3$	56.85	8.11	19.89	56.88	8.14	20.18	A	l	88— 92	7.2	c

[a] A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.
[b] Small n shows colorless needles; l, colorless leaflet; p, colorless plates.
[c] Small a, b, c, d, e, f, and g show the method a, b, c, d, e, f, and g in each compd., respectively, as described in the experimental. Small h show the compd. was prepd. from the corresponding nitro compd. by catalytic reduction over Pd-black.

Table IX. $R_1CONHCH_2CN$ XXVI

Compd. No.	R_1	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	Recryst. solvent[a]	Appearance[b]	mp (°C)	Yield (%)	Method[c]
79	CH_3-	$C_4H_6ON_2$	48.97	6.17	28.56	49.28	5.96	28.45	$CHCl_3$	p	79	88	f
80	$ClCH_2-$	$C_4H_5ON_2Cl$	—	—	21.13	—	—	21.04	H	n	87—89	69	e
81	CH_3CH_2CHBr-	$C_6H_9ON_2Br$	—	—	13.66	—	—	13.24	50% E	n	65—68	81	e
82	$CH_3(CH_2)_3CHBr-$	$C_8H_{13}ON_2Br$	41.22	5.62	—	41.54	5.66	—	B+Ligroin	n	61—64	57	e
83	C_6H_5-	$C_9H_8ON_2$	—	—	17.49	—	—	17.48	M	n	142—143	65	e
84	$C_6H_5CH_2-$	$C_{10}H_{10}ON_2$	68.95	5.79	—	69.07	5.62	—	95% E	n	86—88	87	e
85	$p-NO_2\cdot C_6H_4-$	$C_9H_7O_3N_3$	52.68	3.44	20.48	52.35	3.46	20.57	E	yellow n	142—143	88	e
86	$p-NH_2\cdot C_6H_4-$	$C_9H_9ON_3$	61.70	5.18	23.99	61.57	5.23	24.30	E	n	173—174	96	h
87	$-CH_2-$	$C_7H_8O_2N_4$	46.66	4.48	31.10	46.86	4.61	31.55	E	l	113—114	24	g
88	$HOOCCH_2CH_2-$	$C_6H_8O_3N_3$	46.15	5.16	17.94	45.97	5.04	17.86	$CHCl_3+Et_2O$	n	84—86	89	f
89		$C_{11}H_{11}O_3N_3$	56.65	4.75	18.02	56.45	4.47	18.25	E	n	139	62	g
90		$C_{12}H_{11}O_3N_3$	58.77	4.52	17.14	58.90	4.58	17.09	E	n	189—190	55	g
91	 $HNCO(CH_2)_2CH-$	$C_7H_9O_2N_3$	50.29	5.43	25.14	50.38	5.05	25.36	E	n	76—79	31	g
92	 $CH_3CON(CH_2)_3CH-$	$C_9H_{13}O_2N_3$	55.37	6.71	21.53	55.77	6.38	21.53	A	n	146	37	g
93	$CH_3C(NHCOCH_3)_2-$	$C_9H_{13}O_2N_4$	47.78	6.22	24.77	48.49	6.17	24.85	N	n	204—205	40	d
94	NH_2COCH_2-	$C_5H_7O_2N_3$	42.55	5.00	29.78	42.49	5.14	30.05	P	n	119—121	23	g
95	$CH_2(CN)-$	$C_5H_5ON_3$	48.78	4.09	34.14	48.84	4.15	34.53	E	l	92—93	34	g
96	*trans*-$CH_3CH=CH-$	$C_6H_8ON_2$	58.05	6.50	22.57	58.60	6.13	22.24	*n*-Bu_2O	n	97	74	f
97	$HOCH_2(CHOH)_4-$	$C_8H_{14}O_6N_2$	41.02	6.03	11.96	40.99	6.04	11.78	H+E	n	151	89	[d]
98	$RCO-=CH_3C_6H_4SO_2-$	$C_9H_{10}O_2N_2S$	51.42	4.80	13.33	51.26	4.61	14.27	E	n	136—137	69	e

[a] A is EtOAc; B, benzene; E, EtOH; H, H_2O; M, MeOH; N, MeCN; P, iso-PrOH.

[b] Small n shows colorless needles; l, colorless leaflet; p, colorless plates.

[c] Small a, b, c, d, e, f, and g show the method a, b, c, d, e, f, and g in each compd., respectively, as described in the experimental. Small h show the compd. was prepd. from the corresponding nitro compd. by catalytic reduction over Pd-black.

[d] Prepared from gluconic anhydride and AAN as described in the experiment.

of XXVIII is tedious and the yield is poor and moreover it is impossible to apply this method to synthesis of the amides of dicarboxylic acid, such as malic acid, citric acid, and tartaric acid. Then on the basis of the reactivity of oxazolidone derivatives from aminoacid and chloral,[17] it was considered that chloralides of α-hydroxyacids might be able to use for the synthesis of the amides, and found that the chloralides reacted easily with amines to give the corresponding α-hydroxyacylamides. Glycolic acid chloralide, 2-trichloromethyl-1,3-dioxolane-4-one (XXX), which was easily prepared from XXVII and chloral hydrate by treating with sulfuric acid, reacted with equivalent amount of AAN to give XXIX with about 50% yield. Using two molar equivalents of AAN, the yield rose to about 80%. As a solvent in this reaction, methanol, dioxane, acetonitrile, dichloromethane, and nitromethane were examined, but the yield was not influenced.

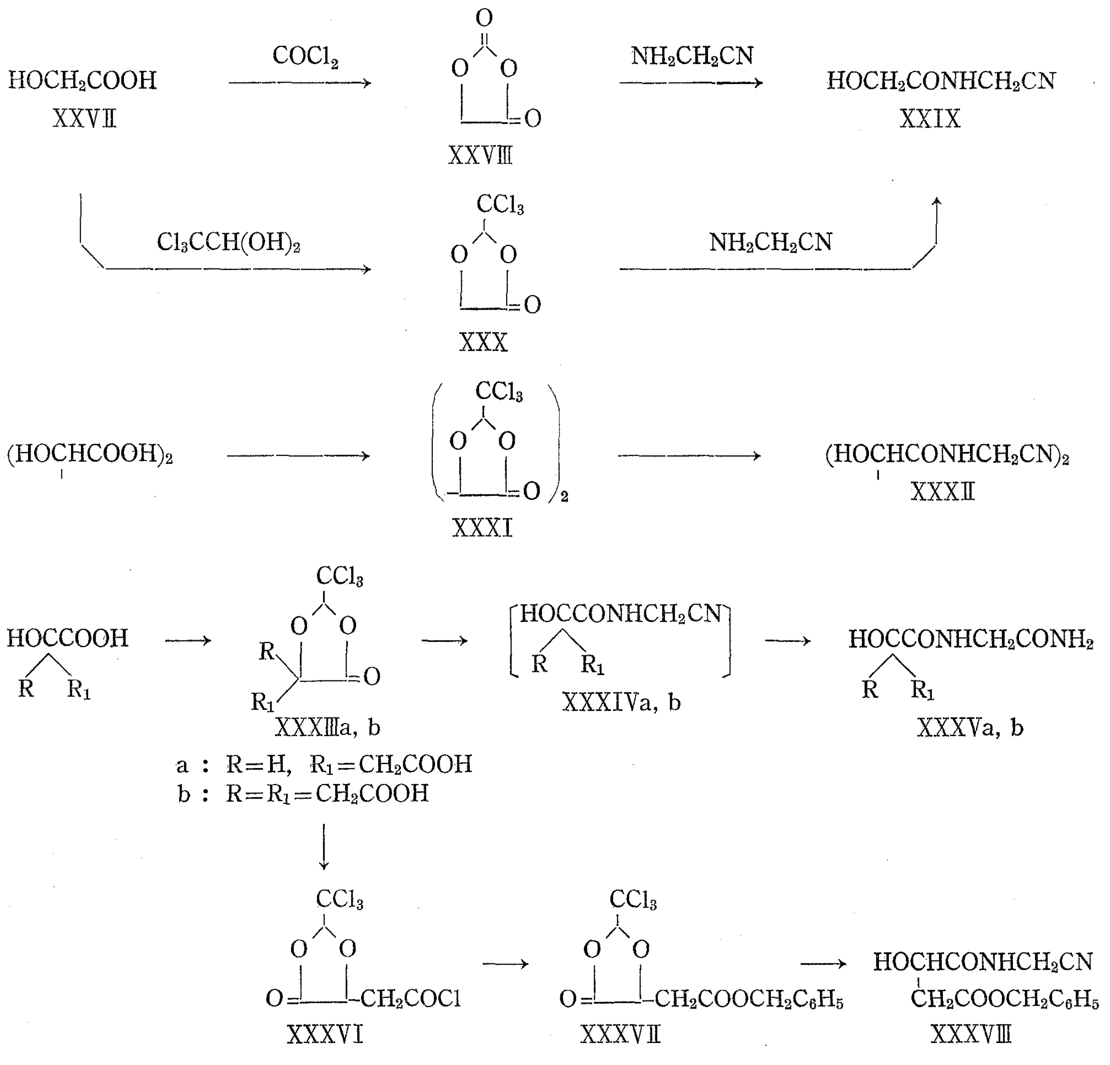


Chart 5

In the same way, tartaryl AAN (XXXII) was obtained from tartaric acid chloralide (XXXI)[18] and AAN. Treatment of the chloralides (XXXIIIa and XXXIIIb)[18] of malic or citric acid with AAN gave the products (XXXIVa and XXXIVb) which were difficult to be purified by crystallization, so attempt to isolate by using amberlit IR-120 to eliminate alkaline media from the reaction mixture resulted in converting XXXIV derivatives to the corresponding

---

[17] E. Dane, R. Heiss, and H. Schäfer, *Angew. Chem.*, **71**, 339 (1959).

[18] N.M. Shah and R. L. Alimchandani, *J. Indian Chem. Soc.*, **11**, 545 (1934) [C. *A*., **29**, 122³ (1935)].

amides (XXXV), accompanied with hydrolysis of the nitrile. The compound (XXXVIII) was obtained from XXXIIIa by conversion via the acid chloride (XXXVI)[19] into the benzylester (XXXVII), followed by treatment with AAN. A detailed study on the reactivities of the chloralides is in progress.

Pharmacological studies on the compounds in this paper will be reported later.

Experimental[20]

N-(N,N-Dimethylglycyl)aminoacetonitrile (II: R=R′=CH_3, Compd. No. 1)——i) I(5.0 g)was added to a cold anhydrous Me_2NH ($-15°$) in a pressure bottle and the mixture was shaken in ice–water for 4 hr, then allowed to reach room temperature and was kept at that temperature for 4 hr. The excess Me_2NH was evaporated *in vacuo*, resulting solid was added to a cold K_2CO_3 saturated aqueous solution and the solution was extracted thoroughly with AcOEt, the extracts were dried over K_2CO_3 and distilled, bp 118—122° (0.25 mmHg), the distillate gradually solidified to colorless needles, mp 65—68°.

ii) A solution of 2.6 g of N–glycyl–AAN acetate in 50 ml of H_2O containing 6 ml of 37% formalin was hydrogenated over Pd–black (0.5 g) at ordinary temperature and pressure, and the reaction mixture was treated by the ordinary method to give the product (1.73 g, 82.3%), mp 66—68°, undepressed by admixture with the sample obtained in i). They also gave identical IR spectra.

N-(N,N-Diethylglycyl)aminoacetonitrile (II: R=R′=C_2H_5, Compd. No. 2)——i) By the same procedure described for method i) of Compd. No. 1, this was obtained from anhydrous Et_2NH and I in 70% yield.

ii) To a solution of 7.3 g of Et_2NH and 8.4 g of $NaHCO_3$ in 100 ml of H_2O was added 13.2 g of I with stirring. After stirring for 3 hr at room temperature, the oil which separated was extracted with $CHCl_3$ and the extracts were washed with water and dried over K_2CO_3. After removal of the solvent, oily residue was distilled at 131—141°/0.25 mmHg to give 12.0 g of the product.

N-(N-Phenylglycyl)aminoacetonitrile (II: R=C_6H_5, R′=H; Compd. No. 3)——A suspension of I(5.3 g), MgO (0.8 g), and aniline (3.7 g) in H_2O (80 ml) was refluxed with stirring for 4 hr. After 3 hr the suspension became clear. The reaction mixture was cooled, and the crystalline solid which separated was collected by filtration, washed with H_2O to give 7.5 g of crystals, which were recrystallized from benzene to give colorless needles , mp 137—140°. In the same way Compd. No. 4—9 in Table I were obtained.

N-(N,N-Dimethylglycyl)glycinamide (III: R=R′=CH_3)——To a 40% aqueous solution (20 g) of Me_2NH cooled to 5° was added 4.0 g of I with stirring at such a rate that the temperature did not rise. When the addition was complete, the ice bath was removed, and the reaction mixture was stirred for 2 hr and allowed to stand overnight, then concentrated under reduced pressure at 40°. The resulting solid was recrystallized from $CHCl_3$ to give 2.6 g colorless scales, mp 137—139°. *Anal.* Calcd. for $C_6H_{13}O_2N_3$: C, 45.27; H, 8.23; N, 26.40. Found: C, 45.01; H, 8.18; N, 26.25. Picrate, yellow needles from EtOH, mp 139—141°. *Anal.* Calcd. for $C_{12}H_{16}O_9N_6$: C, 37.12; H, 4.15; N, 21.65. Found: C, 37.29; H, 4.00; N, 21.56.

N-(N,N-Pentamethyleneglycyl)glycinamide (III: $\genfrac{}{}{0pt}{}{R}{R}{>}N{=}(CH_2)_5N$)——I(3.9 g), piperidine (8.5 g) in H_2O (10 ml) was treated by the same procedure described in the synthesis of III (R=R′=CH_3). Colorless scales (2.1 g) recrystallized from EtOH, mp 164°, were obtained. *Anal.* Calcd. for $C_9H_{17}O_2N_3$: C, 54.25; H, 8.60; N, 21.09. Found: C, 54.03; H, 8.55; N, 21.28. Picrate, yellow needles from EtOH, mp 154—157°. *Anal.* Calcd. for $C_{15}H_{20}O_9N_6$: C, 42.06; H, 4.71; N, 19.62. Found: C, 41.43; H, 4.39; N, 19.36.

N-(N-Phthaloyl-DL-aminoacyl)aminoacetonitriles (VII)——$NaHCO_3$ (0.18 mole) was added to a solution of AAA·½H_2SO_4 (0.06 mole) in H_2O (100 ml). To the solution was added a solution of IV (0.05 mole) in dioxane (50 ml) at 5° with stirring during 30 min. After stirring for 2 hr, the crystalline product was collected, washed with water, and recrystallized (Table II).

N-(N-Phthaloyl-L-leucyl)aminoacetonitrile (L–VII:R=iso-Bu)——This was obtained as same as DL–VII from L–phthaloylleucine[5] ($[\alpha]_D^{18}$ $-23.5°$ (c=4.00, 96% EtOH) (lit.,[5] $[\alpha]_D^{25}$ $-25.2°$ (c=2, 96% EtOH)). Recrystallizations from iso–PrOH afforded pure substance as colorless needles, mp 134—135°, $[\alpha]_D^{18}$ $-13.3°$ (c=4.00, EtOH). *Anal.* Calcd. for $C_{16}H_{17}O_3N_3$: C, 64.20; H, 5.72; N, 14.04. Found: C, 64.31; H, 5.70; N, 14.18.

N-(DL-Aminoacyl)aminoacetonitrile (VIII)——i) VII→VIII: VII (0.01 mole) was added to 30 ml of EtOH containing 0.011 mole of 80% hydrazine hydrate, and the solution was refluxed for 4 hr, concentrated *in vacuo*. The residue was dissolved into 30 ml of H_2O. To this solution, AcOH was added to make pH 5, and the solution was heated at 50° for 15 min, cooled, filtered and the filtrate was concentrated at 50° under reduced pressure, the resulting solid or syrup was recrystallized from a suitable solvent (Table III, Compd. No. 14—17).

---

19) H. Katura, *Nippon Kagaku Zasshi*, 77, 1106 (1956).

20) All melting points were uncorrected.

ii) VI→VII→VIII: VI (0.05 mole), phthalimide–K (0.005 mole) in dimethylformamide (40 ml) was heated on a water bath with stirring for 2.5 hr. The solution was filtered and the filtrate was concentrated under reduced pressure, the resulting syrup (VII) was dissolved into 150 ml of EtOH. Hydrazine hydrate (0.05 mole) was added, and the solution was refluxed for 4 hr, and the mixture was worked up as in i) (Table III, Compd. No. 18 and 19).

N-(N-Benzyloxycarbonylglycyl)aminoacetonitrile (X)——To a solution of AAN·½H_2SO_4(15.7 g) and $NaHCO_3$ (38 g) in H_2O (160 ml), a solution of IX prepared from 27.2 g of cbz.glycine in 50 ml of ether was added at 5° with stirring during 40 min. During the addition the crystalline product separated. When the addition was complete, the ice–bath was removed, and the reaction mixture was stirred until losing the odor of the chloride, and the solid was collected, washed with H_2O, recrystallized from MeOH or H_2O to give 10.6 g of colorless needles, mp 144°. *Anal.* Calcd. for $C_{12}H_{13}O_3N_3$: C, 58.29; H, 5.30; N, 17.00. Found: C, 58.09; H, 5.21; N, 17.32.

The product prepared from VIII (R=H) and cbz.–Cl by the method a) in Chart 4 described later was identical with that obtained in above by mixture melting point determination and IR–spectra comparison.

N-Glycylglycinamide HBr (XI)——To a solution of 36% HBr–AcOH (10 g) was added X (1.2 g) with stirring. After few seconds. CO_2 gas evolved and the solid separated. When the evolution of CO_2 gas stopped, the crystalline product was collected by filtration, washed with AcOH, recrystallized from H_2O–EtOH to give 0.95 g of colorless needles, mp 215—215.5°. *Anal.* Calcd. for $C_4H_{10}O_2N_3Br$: N, 19.91. Found: N, 20.06.

For further confirmation of the structure, this amide was acylated by treating with cbz.–Cl to give cbz.gly.gly.NH_2, colorless needles from MeOH, mp 169°. *Anal.* Calcd. for $C_{12}H_{15}O_4N_3$: C, 54.33; H, 5.70; N, 15.84. Found: C, 54.24; H, 5.48; N, 16.00.

N-L-Leucylaminoacetonitrile (L-VIII:R=iso-Bu)——This was obtained as same as DL–VIII from L–VII (R=iso–Bu). Recrystallizations from MeCN afforded the product as colorless needles, mp 114—116°, $[\alpha]_D^{18}$ +4.5° (c=1.569, H_2O). *Anal.* Calcd. for $C_{10}H_{19}O_3N_3$: C, 52.38; H, 8.35; N, 18.33. Found: C, 52.19; H, 8.55; N, 18.38.

N-β-DL-Aspartyl (and γ-DL-Glutamyl) Aminoacetonitrile (XIV)——A solution of each 0.1 mole of AAN and XII[8] in 150 ml of dioxane was allowed to stand at room temperature for 24 hr. The dioxane was evaporated, the residual syrup was dissolved into 100 ml of H_2O containing 9 g of $NaHCO_3$. To the solution of 0.1 mole of 80% hydrazine hydrate was added, then the mixture was allowed to stand at room temperature for 2 days, made pH 5 with AcOH, the crystalline solid separated was filtered, the filtrate was concentrated under reduced pressure at 60° to give a solid, which was recrystallized from H_2O to colorless needles. They showed a single spot detected by ninhydrin test on paper chromatogram (Toyo filter paper No. 50, Bu—H_2O—AcOH (4:5:1)).

(XIV: $n=2$):*Rf* 0.20. In the ninhydrin determination,[9] 41.87 mg of the probe gave carbondioxide equivalent to 2.51 mg of α–aminocarboxylcarbon; Calcd.: 2.71 mg.

(XIV: $n=1$): *Rf* 0.24. In the ninhydrin determination,[9] 40.35 mg of the sample gave carbondioxide equivalent to 2.70 mg of α–aminocarboxylcarbon; Calcd.: 2.82 mg.

2-Hydroxyimino-5-oxo-6-R-piperazine (XV) and 1-Propyl-(XV: R=H) (XVII)——To a solution of 0.01 mole of VIII (or II: R=H,R′=Pr) and 0.8 g of NH_2OH HCl in 20 ml of H_2O was added 1.7 g of $NaHCO_3$ little by little. The mixture was allowed to stand overnight. The crystalline solid which separated was collected by filtration. The same material was obtained by concentration of the filtrate. The products were summerized in Table V.

2,5-Dioxopiperazine (XVI)——To a solution of 1.2 g XV (R=R′=H) in 15 ml of aq. 1 N HCl was added gradually a solution of 0.8 g of $NaNO_2$ in 5 ml of H_2O with vigorous stirring under cooling with cold water. Crystals began to separate during the addition. After the addition was complete, the stirring was continued for 10 min and the crystals were collected. Recrystallization from water gave 0.55 g of colorless needles, mp 210°, which was not clearly measured because of the sublimation and gradually darkened properties. The product was identical with authentic sample[21] by IR spectra comparison.

N-(N-Phenylglycyl)glycinamidoxime (XVIII)——One point nine grams of II (R=C_6H_5, R′=H; Compd. No. 3) was dissolved in a solution of 0.7 g of NH_2OH HCl and 0.8 g of $NaHCO_3$ in 10 ml of H_2O and 20 ml of EtOH with slight warming. The solution was allowed to stand overnight, then concentrated under reduced pressure and the residue recrystallized from H_2O to give colorless needles, mp 140—142°, weighing 0.8 g. *Anal.* Calcd. for $C_{10}H_{14}O_2N_4$: C, 54.04; H, 6.35; N, 25.21. Found: C, 54.12; H, 6.11; N, 25.05.

5-Oxo-2-phenylcarbamoyloxyimino-piperazine (XIX)——To a solution of 1.2 g of XV (R=H) in aq. 2% NaOH (20 ml) was added 1.0 g of phenylisocyanate at 0—5° with vigorous stirring. After stirring for an hr at same temperature, the crystal which separated was collected and recrystallized twice from AcOEt to give 0.9 g of colorless needles, mp 193° (decomp.). *Anal.* Calcd. for $C_{11}H_{12}O_3N_4$: C, 53.22; H, 4.87; N, 22.57. Found: C, 53.21; H, 4.86; N, 22.53.

---

[21] H.F. Schott, J. B. Larkin, L. B. Rocklavd, and M. S. Dnnn, *J. Org. Chem.*, 12, 490 (1947).

5-Oxo-2-thio-6-R-piperazine (XX)——Through a solution of 0.01 mole of VIII (R=H or R=iso–Bu) dissolved in 6 ml of 14% aq. NH_3, H_2S was passed until saturation. After standing overnight, crystals deposited were collected by filtration.

R=H: Recrystallization from H_2O gave colorless needles, mp 214—220° (decomp.), weighing 0.8 g. *Anal.* Calcd. for $C_4H_6ON_2S$: C, 36.93; H, 4.65; N, 21.53. Found: C, 36.85; H, 4.65; N, 21.45.

R=iso–Bu: Recrystallization from EtOH gave colorless needles, mp 188–189°, weighing 1.4 g. *Anal.* Calcd. for $C_8H_{14}O_3N_2$: C, 51.60; H, 7.58; N, 15.04. Found: C, 51.75; H, 7.39; N, 14.97.

2-Acetamino-5-acetoxypyrazine (XXI)——A suspension of 0.5 g of XV (R=H) in 5 ml of Ac_2O and 2 ml of AcOH was warmed on the water bath at 60° with stirring until the clear solution was obtained. Then the mixture was allowed to stand at room temperature overnight. The resulting darken mixture was evaporated *in vacuo* to deposite the crystalline residue, which was recrystallized from H_2O with active C to give 0.15 g of colorless needles, mp 154—156°. *Anal.* Calcd. for $C_8H_9O_3N_3$: C, 49.23; H, 4.65; N, 21.53. Found: C, 49.29; H, 4.75; N, 21.95.

2-Acetamino-5-hydroxypyrazine (XXII)——Adding 700 mg of XXI to a solution of 4 ml of 10% aq. NaOH with stirring at room temperature, the clear solution was obtained and allowed to stand for an hr. After acidified the solution with AcOH, the white crystals separated were collected and recrystallized from H_2O to give 500 mg of colorless needles, mp 241—245° (sublimed at about 220°). *Anal.* Calcd. for $C_6H_7O_2N_3$: C, 47.05; H, 4.61; N, 27.44. Found: C, 47.00; H, 4.56; N, 27.33.

N-(N-Acylaminoacyl)aminoacetonitrile (XXV: Table VI-VIII)——Method a: To a 10% aq. solution of 1 mole of VIII was added 3 mole of $NaHCO_3$. The solution was cooled to 5°, and 1 mole of acid chloride was added with stirring drop by drop. Lost the odor of the chloride, the precipitate was collected by filtration, washed with H_2O, and recrystallized.

Method b: To a 20% aq. solution of 1 mole of VIII was added 2 mole of acid anhydride with stirring at room temperature for 1 hr. The solution was concentrated *in vacuo* to give the crystalline solid which was recrystallized from a solvent shown in Table V. Compd. No. 56, 57, 65, and 66 separated from the reaction mixture, then were collected by filtration without concentration.

Method c: To a 5—10% solution or suspension of each 1 mole of N–acylaminoacid and AAN in MeCN or Me_2NCHO was added 1.1 mole of DCC with stirring and cooling by ice water. After an hr the ice bath was removed, and the reaction mixture was allowed to stand overnight. To the mixture was added 20 ml of AcOH, and after an hr stirring, dicyclohexylurea separated was filtered off and the filtrate was evaporated to dryness under reduced pressure. The residue was recrystallized from suitable solvent. As a reaction solvent, dimethylformamide was used in the synthesis of Compd. No. 22, 23, 24, 48, and 72. Compd. No. 75 and 77 were prepared in a solution of CH_2Cl_2. In another compds. MeCN was used as solvent.

Method d: To a solution of 0.6 g of AAN in 20 ml of MeCN was added 0.01 mol of 2-methyl-4-substituted-5-oxazolone (XXIV) with stirring and cooling by cooled water. After 3 hr the crystalline solid which separated was collected by filtration and recrystallized.

N-Acylaminoacetonitrile (XXVI: Table IX)——Method e: It was prepared according to the method a) but AAN·$\frac{1}{2}H_2SO_4$ was used instead of VIII.

Method f: It was obtained by the same way described in method b) except that AAN was used instead of VIII.

Method g: It was prepared according to the method c) except that carboxylic acid was used instead of acylaminoacid. As a solvent MeCN was used in Compd. No. 92 and 95. Dimethylformamide was used in Compd. No. 87, 89, 90, 91 and 94.

N-(N-*p*-Aminobenzoylglycyl)aminoacetonitrile (Compd. No. 45)——A solution of 3.1 g of N-(N-*p*-nitrobenzoylglycyl) AAN (Compd. No. 43) in 40 ml of ethyleneglycolmonomethylether hydrogenated at atmospheric pressure and room temperature over Pd–black catalyst (200 mg). Approximate 3 molar equivalent of H_2 was absorbed in about 1.5 hr. The solution was freed of catalyst by filtration, concentrated *in vacuo* to give the crystalline solid which was recrystallized from H_2O to obtain 2.2 g of colorless needles, mp 196—196.5°. By the same way Compd. No. 46 was prepared from Compd. No. 44, No. 63 from 62, No. 69 from 68, and No. 86 from 85.

N-Glycoloylaminoacetonitrile (XXIX)——i) To a solution of 3.3 g of AAN in 20 ml of dioxane was added a solution of 5.9 g of XXVIII in 20 ml of dioxane with stirring and cooling by ice water during 30 min. When the addition was complete, the ice bath was removed, and the reaction mixture was stirred for 2 hr. The solvent was removed *in vacuo* to obtain colorless needles, which was recrystallized from iso–PrOH to give 4.0 g (66.7%) of colorless needles, mp 71.5—72°. *Anal.* Calcd. for $C_4H_6O_2N_2$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.41; H, 5.45; N, 24.76.

ii) A solution of 2.0 g of XXX (This was prepared according to Shah, *et al.*,[18]) mp 41—42° (ether)) and 0.5 g of AAN in 10 ml of dioxane was allowed to stand for 24 hr. The solvent was removed under reduced pressure at 50°. The resulting oil was stirred with 20 ml of ether to give crystalline solid, which was recrystallized from iso–PrOH, weighing 0.6 g (52.6%). When 1.0 g of AAN was used in the above reaction, 0.94 g (82.5%) of XXIX was obtained. This compound was identical with the product obtained in i) by mixture melting point determination and IR spectra comparison.

N,N′-Dicyanomethyl-2,3-dihydroxybutanediamide (XXXII)——A solution of XXXI (4.1 g), AAN (1.3 g) in dioxane (10 ml) was allowed to stand. After 4 hr colorless needles began to separate from the reaction mixture. After 24 hr the crystalline solid was collected by filtration, washed with dioxane, and recrystallized from H_2O to give 1.3 g of colorless needles, mp 188°. *Anal.* Calcd. for $C_8H_{10}O_4N_4$: C, 42.48; H, 4.46; N, 24.77. Found: C, 42.63; H, 4.63; N, 24.81.

N-(3-Carboxy-2-hydroxypropionyl)glycinamide (XXXVa)——To a solution of 1.5 g of AAN in 20 ml of H_2O was added 2.6 g of XXXIIIa. The suspension was warmed on a water bath until it became clear, then the water bath was removed and the solution was allowed to stand for 20 hr, treated with amberlite IR—120, concentrated *in vacuo*. The resulting oil crystallized by adding EtOH and stirring. It was recrystallized from EtOH to give 1.1 g of colorless needles, mp 161—162° (decomp.). *Anal.* Calcd. for $C_6H_{10}O_5N_2$: C, 37.90; H, 5.30; N, 14.73. Found: C, 38.14; H, 5.32; N, 14.88. Combined water in this product was not appreciated by Karl Fischer reagent.

N-(3-Carboxy-2-carboxymethyl-2-hydroxypropionyl)glycinamide (XXXVb)——AAN (1.5 g) and XXXIIIb (3.2) were treated by the same manner described in XXXIVa. Colorless needles (0.5 g, 21.7%), mp 178—189° (decomp.), being recrystallized from EtOH was obtained. *Anal.* Calcd. for $C_8H_{12}O_7N_2$: C, 38.71; H, 4.87; N, 11.29. Found: C, 38.82; H, 4.95; N, 11.53. Combined water in the product was not appreciated by Karl Fischer reagent.

2-Trichromethyl-1,3-dioxolane-4-one-5-acetic Acid Benzylester (XXXVII)——A solution of XXXVI (prepared from 5.0 g of XXXIIIa)19) and 3.0 g of benzylalcohol in 25 ml of benzene was refluxed on a water bath for 5 hr. The reaction mixture was cooled, and the crystals which separated were collected and recrystallized from benzene to give colorless plates, mp 146—148°, weighing 5.1 g. *Anal.* Calcd. for $C_{13}H_{11}O_5Cl_3$: C, 44.15; H, 3.13. Found: C, 44.28; H, 3.40.

N-(3-Benzyloxycarbonyl-2-hydroxypropionyl)aminoacetonitrile (XXXVIII)——A solution of 2.5 g of XXXVII and 2.0 g of AAN in 30 ml of Me_2NCHO was allowed to stand for 24 hr. After concentration of the reaction solution, the resulting solid was twice recrystallized from H_2O to give 0.9 g of colorless needles, mp 98°. *Anal.* Calcd. for $C_{13}H_{14}O_4N_2$: C, 59.53; H, 5.34; N, 10.68. Found: C, 59.59; H, 5.25; N, 10.83.

N-Gluconylaminoacetonitrile (Compd. No. 97)——This was prepared from gluconic acid anhydride and AAN according to the method of Fieser, *et al.*22)

Acknowledgement The authors are very grateful to the members of the Analysis Room of this laboratory for elemental and spectral analysis.

---

22) M. Fieser, L. F. Fieser, E. Toromahoff, Y. Hirata, H. Heymann, M. Tefft, and S. Bhattacharya, *J. Am. Chem. Soc.*, **78**, 2827 (1956).