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174. Issei Iwai and Norio Nakamura : Studies on Acetylenic Compounds. XLIV.*1 Synthesis of 3-Aminoisoxazoles and 3-Hydroxyisoxazoles (3-Isoxazolones).

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Syntheses of a number of 4-, 5-amino- and 3-alkylamino- or arylamino-isoxazoles have been reported, but fewer methods have been known for the synthesis of 3-aminoisoxazoles[1~7] whose sulfonamides show antibacterial activity. Recently, 4-substituted 3-aminoisoxazoles were prepared by the reaction of hydroxylamine with α-substituted α,β-dihalopropionitriles or β-haloacrylonitriles.[8] As for 3-isoxazolones, which exist in hydroxy-form,[9,10] few synthetic methods have been reported,[5,9~14] while a lot of 5-isoxazolones have been synthesized.

In this paper the authors wish to report a new convenient method for the syntheses of 3-amino- and 3-hydroxyisoxazoles from acetylenic nitriles and esters, respectively.

β-Bromocinnamonitrile (I) obtained by the addition of cyanogen bromide to phenylacetylene[15] reacted with an alcoholic solution of hydroxylamine to afford 3-phenyl-5-aminoisoxazole (II) (13.8%) of m.p. 109~110° and 3-amino-5-phenylisoxazole (III) (5.3%) of m.p. 137~138°, which were identified with the corresponding authentic samples[4,16] by mixed melting point test and comparison of their infrared spectra. In a 1:1 mixture of aqueous 10% sodium hydroxide and ethanol, I and hydroxylamine afforded only III (94%) after standing overnight at 30°. Obviously, the presence of alkali played an important role in the reaction mode to afford 3-amino-5-phenylisoxazole (III). Under the same conditions, β,4-dibromocinnamonitrile[15] reacted with hydroxylamine to afford 3-amino-5-(*p*-bromophenyl)isoxazole (IV) of m.p. 147~149°.

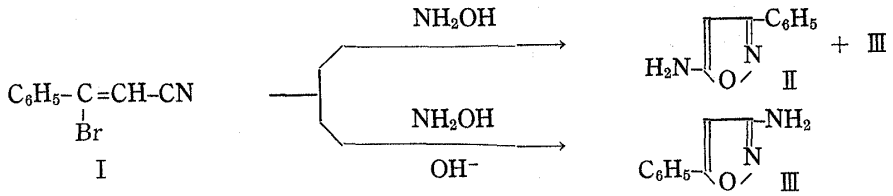

Chart 1.

*1 Part XLIII : This Bulletin, **14**, 92 (1966).
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1) A. Quilico : Gazz. chim. ital., **61**, 970 (1931) (C. A., **26**, 2978 (1932)).
2) *Idem* : *Ibid.*, **76**, 255 (1946) (C. A., **42**, 1262 (1946)).
3) H. Kano, K. Ogata : Shionogi's Ann. Rep., **7**, 1 (1957).
4) H. Kano, I. Kitsukawa, T. Makizumi, S. Takahashi, H. Ogata : Jap. Pat., 303,134 (1962); Brit. Pat., 875,458 (C. A., **56**, 8720 (1962)).
5) H. D. Stachel : Chem. Ber., **96**, 1088 (1963).
6) B. Tornetta : Ann. Chim. (Rome), **53**, (3) 244 (1963).
7) J. T. Plati, W. Wenner : Fr. Pat., 1,363,643 (1964) (C. A., **61**, 14678 (1964)).
8) Hoffman-La Roche Co. A-G : Neth. Pat., 6,408,283 (1965).
9) P. Bravo, G. Gaudiano, A. Quilico, A. Ricca : Gazz. chim. ital., **91**, 47 (1961).
10) A. J. Boulton, A. R. Katritzky, A. Majid Hamid, S. Øksne : Tetrahedron, **20**, 2835 (1964).
11) S. Cabiddu, G. Gaudiano, A. Quilico : Gazz. chim. ital., **92**, 501 (1962).
12) R. Uhlenhuth : Ann., **296**, 33 (1897).
13) L. Bauer, C. N. V. Nambury : J. Org. Chem., **26**, 4917 (1961).
14) A. R. Gagneux, F. Häfliger, C. H. Eugster, R. Good : Tetrahedron Letters, **1965**, 2077.
15) I. Iwai, T. Iwashige, Y. Yura, N. Nakamura, K. Shinozaki : This Bulletin, **12**, 1446 (1964).
16) A. Obrégia : Ann., **266**, 324 (1891).

In the alkaline reaction mixture, an acetylenic nitrile would be afforded as an intermediate by dehydrobromination of the β-bromocinnamonitrile. Therefore, the authors investigated the reaction of acetylenic nitriles and esters with hydroxylamine in the presence of alkali.

On the reaction of α-acetylenic nitriles with hydroxylamine, only one work by Moureu and Lazennec has been found. They have reported that phenylpropiolonitrile reacted with an equimolecular mixture of hydroxylamine hydrochloride and sodium ethoxide to yield 3-phenyl-5-amino-isoxazole (II).[17] It was found, however, that in the presence of alkali the reaction of phenylpropiolonitrile with hydroxylamine proceeded in another way to afford only 3-amino-5-phenylisoxazole in 85% yield. Similarly 3-amino-5-(*p*-methoxyphenyl)isoxazole (V) of m.p. 171~172° was obtained from *p*-methoxyphenylpropiolonitrile under the same conditions.

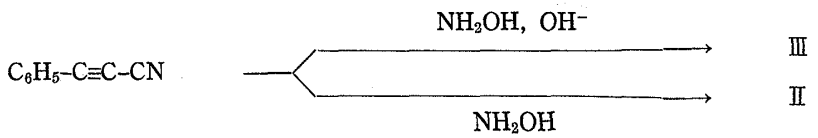

Chart 2.

In correspondence to the result of Moureu and Lazennec, reaction of tetrolonitrile with a stoichiometric mixture of hydroxylamine hydrochloride and aqueous 10% sodium hydroxide gave 3-phenyl-5-aminoisoxazole (VI) of m.p. 84~85°, whose infrared spectrum was identical with that of an authentic sample.[18] In the presence of excess of alkali, however, the reaction gave a crystalline substance ($C_4H_6ON_2$, m.p. 45~52°, 90% yield), which was found to be a mixture of 3-amino-5-methylisoxazole (VII) and VI by the comparison of the nuclear magnetic resonance spectrum with that of VI (Fig. 1). In the nuclear magnetic resonance (NMR) spectra, signals appeared at 2.14, 2.27, 4.20, 4.70, 4.97, and 5.56 p.p.m. for the mixture, and at 2.14, 4.70 and 4.97 p.p.m. for VI in deuterochloroform. Therefore, the signals at 2.27 (doublet, J=1 c.p.s.-CH_3), 4.20 (broad, -NH_2) and 5.56 p.p.m. (doublet, J=1 c.p.s. C_4-H) were attributed to VII. On the intensity of the signals due to the methyl groups, the ratio of VI to VII was found

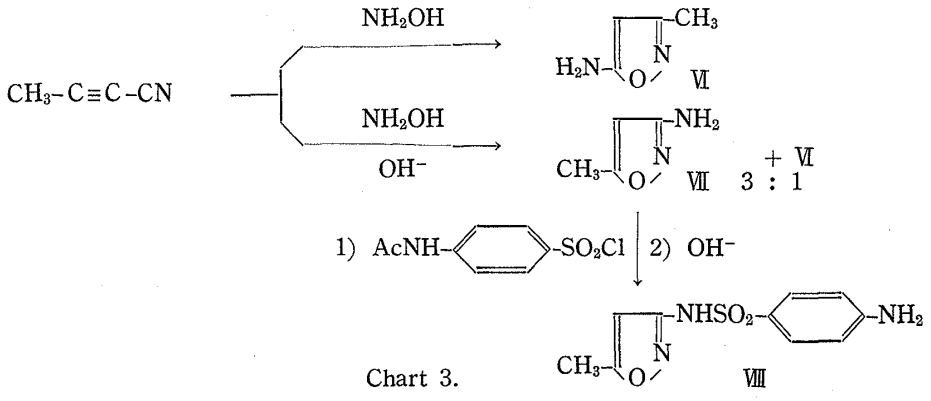

Chart 3.

to be 1:3. This mixture was treated with N-acetylsulfanilyl chloride to give only the sulfonamide of VII under the conditions employed. Subsequent hydrolysis afforded 3-sulfanilamido-5-methylisoxazole (m.p. 167~168°) (VIII), which showed no depression in melting point on admixture with an authentic sample.[3]

17) C. Moureu, I. Lazennec : Bull. soc. chim. France, [4] **1**, 1079 (1907).
18) G. Show, G. Sugowdy : J. Chem. Soc., **1954**, 665.

Similarly, propiolonitrile and hydroxylamine gave 5-aminoisoxazole (IX) of m.p. 76~78° (benzoate (X), m.p. 134~135°) in the absence of excess of alkali, and 3-aminoisoxazole (XI) (benzoate (XII), m.p. 148~149° (lit. m.p. 149°[01])) under alkaline conditions. The yields of IX and XI were 44 and 63%, respectively, based on the amount of propiolamide which had been dehydrated with phosphorous pentoxide to the starting propiolonitrile.[19] By an usual method XI was converted to 3-sulfanilamidoisoxazole (XIII) of m.p. 124~126°.

The NMR spectra of IX and XI were reasonable for their structures as shown in Fig. 2. Comparing these spectra with those of VI and VII, peaks at 7.3~8.0 p.p.m. were assigned to C_3-H of IX and C_5-H of XI. Infrared and ultraviolet absorption bands of new aminoisoxazoles are listed in Table I.

All of the papers[17,22~24] on the reaction of α-acetylenic esters with hydroxylamine have described syntheses of 5-isoxazolones :

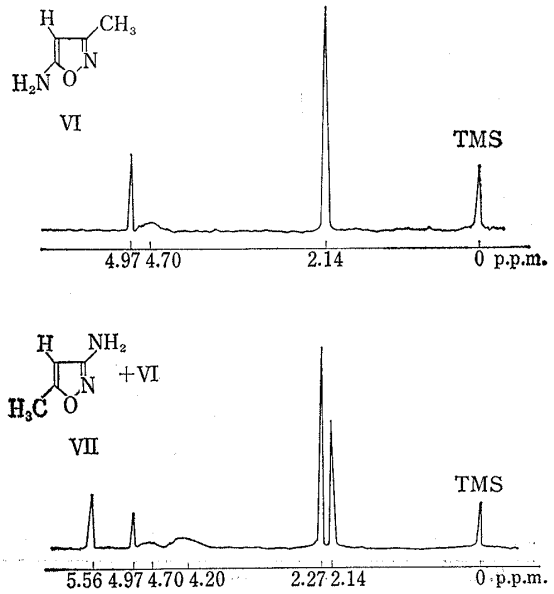

Fig. 1. Nuclear Magnetic Resonance Spectra (60 Mc.) of 3-Methyl-5-aminoisoxazole (VI) and the mixture of 3-Amino-5-methylisoxazole (VII) and VI in Carbon Tetrachloride using Tetramethylsilane as the Internal Standard

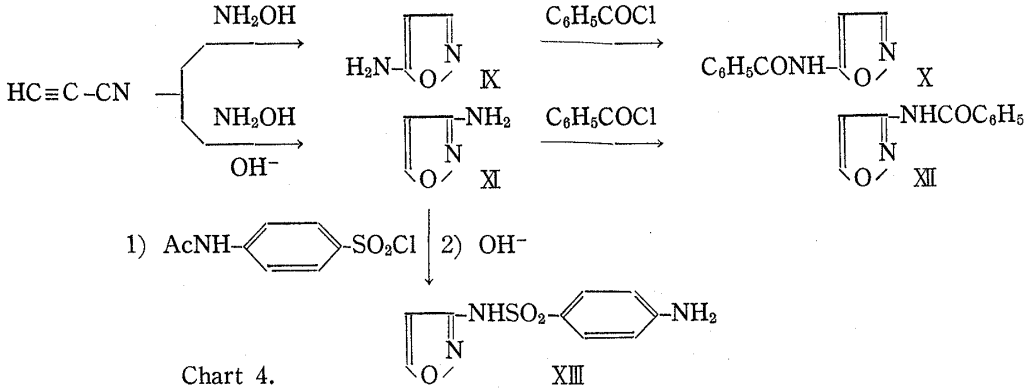

Chart 4.

TABLE I.[20,21] Infrared and Ultraviolet Spectra of Aminoisoxazoles

No.	Substituent 3	Substituent 5	IR $\nu_{max}^{CHCl_3}$ cm^{-1}: NH_2 stretching	IR: NH_2 acissor and isoxazole ring modes[20,21]	UV λ_{max}^{EtOH} mμ (ε)
IV	NH_2	*p*-Br-C_6H_4	3485, 3390	1623, ——, 1515, 1468	267 (23,900)
V	〃	*p*-CH_3O-C_6H_4	3480, 3390	1623, 1603, ——, 1466	272 (25,130)
XI	〃	H	3450, 3355	1618, 1584, 1488, 1443	228.5(2,830)
IX	H	NH_2	3520, 3425	1634, 1592, 1502, ——	245.5(15,440)

19) C. Moureu, I. Lazennec : Ann. chim. Fr., [9], **409**, 53 (1920).
20) A. R. Katritzky, A. J. Boulton : Tetrahedron, **12**, 51 (1961).
21) *Idem* : Spectrochim. Acta, **17**, 238 (1961).
22) Olivieri-Mandalà : Gazz. chim. ital., **40**, **I**, 126 (1910).
23) D. W. Verwoerd, H. Kohlhage, W. Zillig : Nature, **192**, 1038 (1961).
24) H. Schuster : J. Mol. Biol., **3**, 447 (1961).

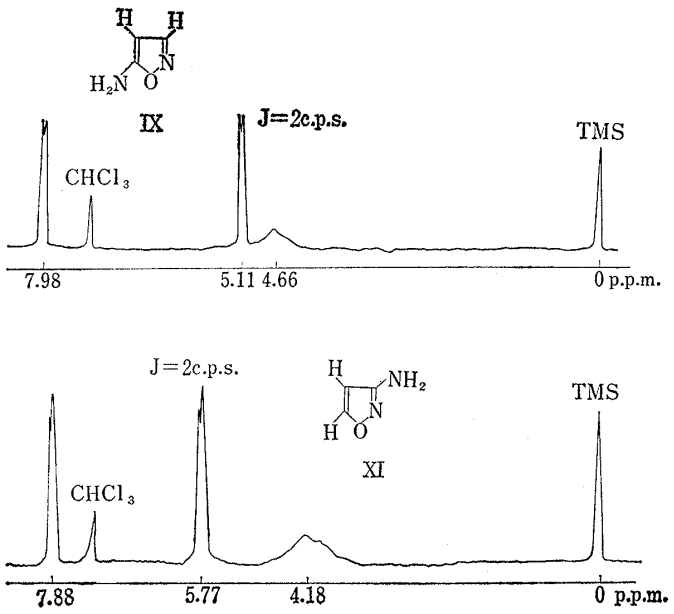

Fig. 2. Nuclear Magnetic Resonance Spectra (60 Mc.) of 5-Aminoisoxazole (IX) and 3-Aminoisoxazole (XI) in Deuterochloroform using Tetramethylsilane as the Internal Standard

slightly alkaline alcoholic solution of hydroxylamine and ethyl tetrolate yielded an autocondensation product of 3-methyl-5-isoxazolone, *i.e.* 3-methyl-4-(3-methyl-5-isoxazolinylidene)-5-isoxazolone[22,25]; the compound obtained[26] by treatment of ethyl phenylpropiolate with an equimolecular mixture of hydroxylamine hydrochloride and sodium ethoxide and described[27] as 5-phenyl-3-isoxazolone has been proved to be 3-phenyl-5-isoxazolone[17]; ethyl propiolate and hydroxylamine in an aqueous solution (pH 10) afforded 5-isoxazolone which was stable only in the anionic form.[23,24]

Ethyl phenylpropiolate, however, reacted with hydroxylamine in the presence of alkali to yield 3-hydroxy-5-phenylisoxazole (XIV) of m.p. 163～165°, whose infrared spectrum was

Table II. Infrared Spectra of Tetrolohydroxamic Acids

No.	m.p. (°C)	Phase	ν_{max} cm^{-1}
XIX	115～117	KBr	3195, 3058, 2994(broad), 2873, 2273, 2232, 1645, 1621, 1570, 1479, 1330, 1124, 1025, 871, 758
XX	143～144	〃	3195, 2780(broad), 2262, 2227 1612, 1545, 1462, 1382, 1119, 1025, 870, 769

identical with that of an authentic sample prepared from 3-methoxy-5-phenylisoxazole according to Quilico's method.[9,11] Similarly, 5-(*p*-nitrophenyl)- (XV), 5-(*p*-chlorophenyl)- (XVI) and 5-(*p*-methoxyphenyl)-3-hydroxyisoxazole (XVII) were synthesized from the corresponding acetylenic esters (see Table III). All of their infrared spectra showed no carbonyl band, as in the case of other 3-hydroxyisoxazoles.[9,10,12]

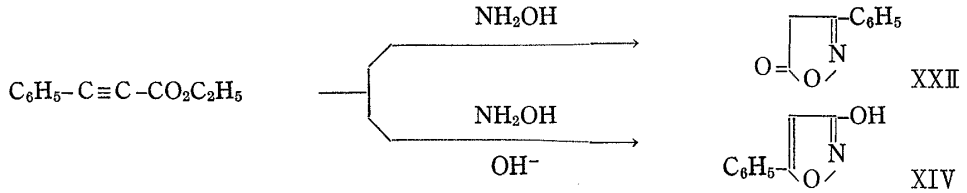

Chart 5.

At 25～30°, methyl tetrolate reacted with hydroxylamine in the presence of excess of alkali to give a crystalline compound of m.p. 84～85° (XVIII). On the other hand, at 5～10° the same mixture afforded two isomers of tetrolohydroxamic acid, XIX of m.p.

25) J. J. Donleavy, E. E. Gilbert: J. Am. Chem. Soc., **59**, 1072 (1937).
26) S. Ruhemann, H. E. Stapleton: J. Chem. Soc., **77**, 239 (1900).
27) S. Ruhemann, A. V. Cunnington: *Ibid.*, **75**, 957 (1899).

115～117° and XX of m.p. 143～144°. The infrared spectra of both XIX and XX showed acetylenic bands as shown in Table II. These hydroxamic acids were considered to be tautomers (A) and (B).[28~30] Further treatment of both XIX and XX with 10% sodium hydroxide solution at 30° gave XVIII. Therefore, the structure of XVIII was proved to be 3-hydroxy-5-methylisoxazole.

Under the same conditions as described above, ethyl propiolate was converted to 3-hydroxyisoxazole (XXI) of m.p. 98～99°, whose infrared and neuclear magnetic resonance spectra were similar to those of XVIII.

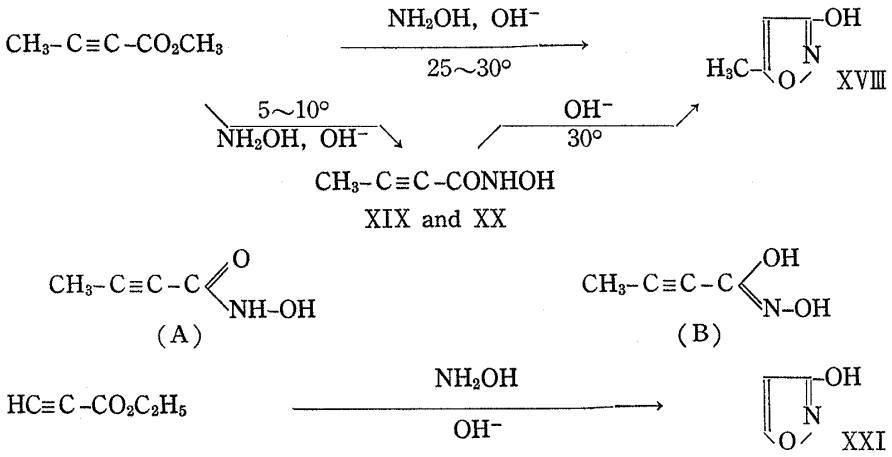

Chart 6.

Table III. 3-Hydroxyisoxazoles

No.	R	m.p. (°C)	Yield (%)	UV λ_{max}^{EtOH} mμ (ε)
XIV	C_6H_5	163～165	87	260(19,070)
XV	p-NO_2-C_6H_4	241～243(decomp.)	77	225(11,910), 307.5(14,240)
XVI	p-Cl-C_6H_4	220～221(〃)	52	266(20,800)
XVII	p-CH_3O-C_6H_4	212～214(〃)	48	273.5(23,530)
XVIII	CH_3	84～ 85	92	<220
XXI	H	98～ 99	59	<220

Infrared absorption bands, characteristic of isoxazole ring,[20,21] are listed in Table IV for new 3-hydroxy isoxazoles. All of the 3-hydroxyisoxazoles showed strong broad absorptions in 3200～2500 cm^{-1} region due to strong hydrogen bonding in carbon tetrachloride, chloroform, nujol mull and potassium bromide pellets. In dilute carbon tetrachloride solution, 3-hydroxyisoxazole (XXI) at 0.015M (5 mm. cell) showed a weak but sharp band (ε^a=6) at 3570 cm^{-1}, which became stronger (ε^a=10) at 0.0015M (50 mm. cell). For 3-hydroxy-5-methylisoxazole (XVIII), a similar absorption was found at 3570 cm^{-1} (ε^a=3 at 0.015M and ε^a=7 at 0.0015M) in carbon tetrachloride. These bands were assigned to the free hydroxyl group. A similar absorption band has been observed at 3550 cm^{-1} for 3-hydroxy-4,5-dimethylisoxazole.[10]

28) H. L. Yale : Chem. Rev., **33**, 209 (1943).
29) F. Mathis : Bull. soc. chim. France, **33**, D9 (1953).
30) *Idem* : Ann. Fac. Sc. Univ. Toulouse, Sci. Phys., **25**, 111 (1961) (C. A., **60**, 11885f (1964)).

Table IV. Infrared Spectra of 3-Hydroxyisoxazoles

No.	5-Substituent	Phase	Ring stretching modes[20,21] (cm^{-1})	Ring deformation mode[20,21] (cm^{-1})
XV	*p*-NO_2-C_6H_4	Nujol	1634, 1543, 1464, ——	944
XVI	*p*-Cl-C_6H_4	〃	1631, 1536, 1486, ——	941
XVII	*p*-CH_3O-C_6H_4	〃	1632, 1536, 1470, ——	943
XVIII	CH_3	CCl_4	1628, 1519, ——, 1445	933
XXI	H	$CHCl_3$	1604, 1524, 1484, ——	943

Nuclear magnetic resonance data are shown in Table V. The sharp peaks at about 11~12 p.p.m. in carbon tetrachloride, which is absent in deuterium oxide, confirms that XVIII and XXI exist in hydroxy-form.

Table V. Nuclear Magnetic Resonance Spectra of 3-Hydroxyisoxazoles

No.	Solvent	Substituent and Chemical Shifts (p.p.m.)[a] 3		4		5	
XIV	$CDCl_3$	OH	9.84	H	6.20	C_6H_5	7.2~8.0
	$(CD_3)_2SO$	〃	11.40(broad)	〃	6.58	〃	7.3~8.0
XV	〃	〃	11.76(〃)	〃	6.84	*p*-NO_2-C_6H_4	7.8~8.6
XVI	〃	〃	10.40(〃)	〃	6.60	*p*-Cl-C_6H_4	7.70(center, A_2B_2 quartet)
XVII	〃	〃	——[b]	〃	6.38	*p*-CH_3O-C_6H_4	3.82(-OCH_3)
							7.40(center, A_2B_2 quartet)
XVIII	CCl_4	〃	11.92	〃	5.64	CH_3	2.34
	D_2O	〃	——	〃	5.84	〃	2.34
	$(CD_3)_2SO$	〃	——[b]	〃	5.75	〃	2.26
XXI	$CDCl_3$	〃	10.88	〃	6.10[c]	H	8.16[c]
	D_2O	〃	——	〃	6.18[c]	〃	8.41[c]
	$(CD_3)_2SO$	〃	11.20(broad)	〃	6.10[c]	〃	8.54[c]

a) As the internal standard, 3-trimethylsilyl-1-propanesulfonic acid sodium salt was used in deuterium oxide and tetramethylsilane in other solvents.
b) In the case of XVII and XXI, the 3-hydroxyl peaks were absent in hexadeuterodimethylsulfoxide. See Ref. 10).
c) doublet, J=2 c.p.s.

Isolation of hydroxamic acids XIX and XX indicates that 3-hydroxyisoxazoles were formed from α-acetylenic esters *via* acetylenic hydroxamic acids in the presence of alkali. In fact, free phenylpropiolic acid, which was unfavorable for hydroxamic acid formation, gave only 3-phenyl-5-isoxazolone (XXII) under the same conditions. Similarly, acetylenic amidoximes (XXIII) would be intermediates for the formations of 3-aminoisoxazoles from α-acetylenic nitriles.

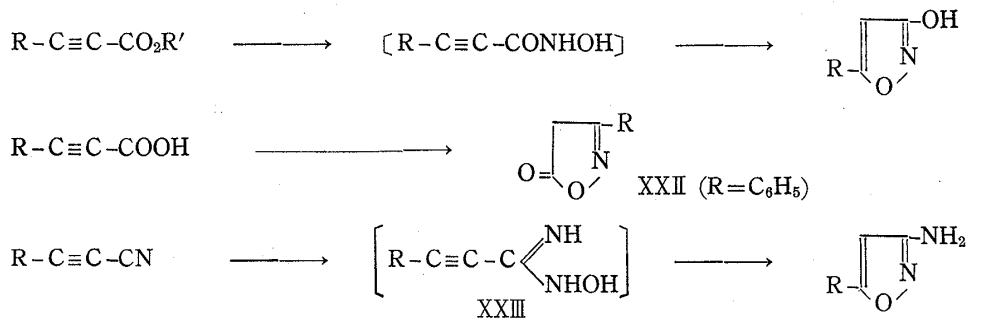

Chart 7.

From these results, it is concluded that the addition of hydroxylamine to a triple bond is slower than hydroxamic acid or amidoxime formation in the presence of alkali, while in the absence of excess of alkali hydroxylamine reacted faster with the triple bond than with the ester or nitrile group.

Experimental*3

Reaction of β-Bromocinnamonitrile (I) with Hydroxylamine under a Neutral Condition——To a mixture of β-bromocinnamonitrile (I) (3.00 g., 0.014 mole), hydroxylamine hydrochloride (2.98 g., 0.043 mole) and 50 ml. of MeOH was added a solution of sodium methoxide prepared from sodium (0.93 g., 0.040 mole) and 30 ml. of MeOH during 2 hr. at 0°. After stirred for 1 hr. at 0°, 2 hr. at 20° and 48 hr. at 35~40°, the solvent was evaporated. The residue was extracted with ether. The extract was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue was dissolved in benzene-acetone (10:1) and chromatographed on Al_2O_3 (Woelm, Grade III, 100 g.). The same solvent eluted 0.320 g. (13.8%) of 3-phenyl-5-aminoisoxazole (II), m.p. 110~111° (from hexane-benzene), which showed no depression in m.p. on admixture with an authentic sample prepared by Obrégia's method.[16] *Anal.* Calcd. for $C_9H_8ON_2$: C, 67.48; H, 5.03; N, 17.49. Found: C, 67.42; H, 5.09; N, 17.32. Benzene-acetone (5:1) eluted 0.122 g. (5.3%) of 3-amino-5-phenylisoxazole (III), m.p. 137~138°, which was identified by mixed melting point test and IR spectrum comparison with an authentic sample prepared from 5-phenylisoxazole-3-carboxamide according to Kano's method.[4] *Anal.* Calcd. for $C_9H_8ON_2$: C, 67.48; H, 5.03; N, 17.32. Found: C, 67.60; H, 5.15; N, 17.64.

3-Amino-5-phenylisoxazole (III) from (I)——To a solution of β-bromocinnamonitrile (1.056 g., 0.005 mole) in EtOH (2 ml.) was added at once a mixture of hydroxylamine hydrochloride (1.8 g., 0.026 mole), 10% NaOH (15.5 ml., 0.038 mole) and EtOH (20 ml.). After standing overnight, the reaction mixture was extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. Recrystallization from aq. EtOH gave white needles of 3-amino-5-phenylisoxazole (III) m.p. 137~138°, whose IR spectrum was superimposable on that of an authentic sample.[4] Yield, 0.766 g. (94%).

3-Amino-5-(*p*-bromophenyl)isoxazole (IV)——A solution of β,4-dibromocinnamonitrile[15] (0.816 g., 0.0028 mole) in 10 ml. of EtOH was mixed with hydroxylamine hydrochloride (1.10 g., 0.016 mole) in 10 ml. of 10% NaOH (0.025 mole). After standing overnight, the mixture was extracted three times with ether. The ethereal layer was extracted with 10% HCl solution. After neutralization with aq. NaOH, yellow precipitate was taken up in ether. After evaporation of the solvent, the residue was recrystallized from aq. MeOH to give yellow prisms of 3-amino-5-(*p*-bromophenyl)isoxazole (IV), m.p. 147~149°. Yield, 0.390 g. (57%). *Anal.* Calcd. for $C_9H_7ON_2Br$: C, 45.39; H, 2.98; N, 11.77. Found: C, 45.23; H, 2.98; N, 11.73.

Synthesis of III from Phenylpropiolonitrile——A solution of phenylpropiolonitrile (1.27 g., 0.01 mole) in 20 ml. of EtOH was mixed with hydroxylamine hydrochloride (3.6 g., 0.05 mole) dissolved in 28 ml. of 10% NaOH (0.07 mole). After stirred for 24 hr. at 30°, the mixture was extracted with ether. The extract was washed with 5% $NaHCO_3$, H_2O, dried over Na_2SO_4 and the solvent was evaporated. The oily residue was taken up in 10% HCl, and crude 3-amino-5-phenylisoxazole (III) (1.36 g., 85%) was precipitated by adding 10% NaOH. Recrystallization from aq. EtOH gave white needles of III, m.p. 137~138°.

***p*-Methoxyphenylpropiolonitrile**——The following method is an analogue to Grignard's.[31] To a solution of cyanogen chloride (12.2 g.) in 150 ml. abs. ether was added a solution of a Grignard reagent prepared from *p*-methoxyphenylacetylene (25.8 g.), magnesium (4.9 g.) and 120 ml. of tetrahydrofuran, keeping the inner temperature at −15~−5°. After stirred at 0° for 2 hr. and standing overnight at room temperature, the mixture was poured into 7% HCl and extracted three times with ether. The extract was washed with 5% NaOH, 5% H_2SO_4, 10% Na_2CO_3, satd. aq. NaCl and dried over Na_2SO_4. After evaporation of ether, the residue (35.3 g.) was dissolved in hexane and chromatographed on Al_2O_3 (Woelm, Grade II, 600 g.). The same solvent eluted 1.527 g. of *p*-methoxyphenylpropiolonitrile after halogen-containing fractions. Recrystallization from hexane gave white needles of m.p. 78.5~80°. IR ν_{max}^{Nujol} cm^{-1}: 2266 (-C≡C-), 2137 (-CN), 1267, 1029 (-C-O-C-). UV λ_{max} mμ (ε): 289 (26,400), 294 (28,200). *Anal.* Calcd. for $C_{10}H_7ON$: C, 76.82; H, 4.49; N, 8.91. Found: C, 76.82; H, 4.42; N, 8.87.

3-Amino-5-(*p*-methoxyphenyl)isoxazole (V)——To a mixture of hydroxylamine hydrochloride (1.25 g., 0.018 mole) and 10% NaOH (25 ml., 0.062 mole) was added a solution of *p*-methoxyphenylpropiolonitrile (0.94 g., 0.006 mole) in 25 ml. of EtOH. After standing overnight the mixture was extracted with ether. The extract was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from aq. EtOH to give white needles of 3-amino-5-(*p*-methoxyphenyl)isoxazole (V), m.p. 171~172°. Yield, 0.54 g. (41%). *Anal.* Calcd. for $C_{10}H_{10}O_2N$: C, 63.15; H, 5.30; N, 14.73. Found: C, 62.88; H, 5.37; N, 14.54.

*3 All melting points are uncorrected. UV spectra were taken in EtOH.

31) V. Grignard, C. Courtot: Bull. soc. chim. France, [4] **17**, 230.

Tetrolonitrile——Analogously to Moureu's method[21] for propiolonitrile, tetrolonitrile was synthesized from tetrolamide: a mixture of tetrolamide (1.527 g.), P_2O_5 (2.0 g.) and sea sand (2.0 g.) was heated on an oil bath at 150~160°. Immediately was distilled 0.892 g. (74%) of tetrolonitrile, b.p_{146} 59~60° (lit. b.p_{146} 60.5~61°[32]). IR: ν_{max}^{film} cm^{-1}: 2320, 2268 (-C≡C-), 2155 (-CN). *Anal.* Calcd. for C_4H_3N: C, 73.83; H, 4.65; N, 21.53. Found: C, 73.93; H, 4.75; N, 21.42.

3-Methyl-5-aminoisoxazole (VI)——To a mixture of hydroxylamine hydrochloride (1.668 g., 0.024 mole) and 10% NaOH (8.8 ml. 0.022 mole) was added a solution of tetrolonitrile (1.300 g., 0.020 mole) in 6 ml. of EtOH. The reaction mixture, which was neutral to litmus, was stirred for a night, saturated with Na_2SO_4 and extracted three times with ether. The extract was dried over Na_2SO_4, filtered and the solvent was evaporated. The residue was recrystallized from benzene to afford white needles of 3-methyl-5-aminoisoxazole (VI), m.p. 84~85°, which was identical with an authentic sample in mixing melting point test. Yield, 1.707 g. (86%). *Anal.* Calcd. for $C_4H_7ON_2$: C, 48.97; H, 6.17; N, 28.56. Found: C, 49.03; H, 6.20; N, 28.79.

Reaction of Tetrolonitrile with Hydroxylamine in the presence of Sodium Hydroxide——To a mixture of hydroxylamine hydrochloride (1.3 g., 0.019 mole) and 10% NaOH (15 ml., 0.0375 mole) was added a solution of tetrolonitrile (0.820 g., 0.0126 mole) in 20 ml. of EtOH. After standing overnight at 30°, the mixture was saturated with NaCl and extracted three times with ether. The ethereal layer was dried over Na_2SO_4, filtered and the solvent was evaporated. The residue was purified with benzene to give a white crystalline solid of m.p. 45~52°, which was a 3:1 mixture of 3-amino-5-phenylisoxazole (VII) and VI. (See Fig. 1.) Yield, 1.113 g. (90%). *Anal.* Calcd. for $C_4H_6ON_2$: C, 48.97; H, 6.17; N, 28.56. Found: C, 48.88; H, 6.15; N, 28.77.

3-Sulfanilamido-5-methylisoxazole (VIII)——To a solution of the 3:1 mixture of VII and VI (4.5 g.) in pyridine (25 ml.) was added N-acetylbenzenesulfanilyl chloride (10.0 g.). After stirring for 1 hr., the reaction mixture was poured into 200 ml. of H_2O. Brown crystalline precipitate was collected, dried and recrystallized from large amount of EtOH to give colorless prisms of 3-(N-acetylbenzenesulfanilamido)-5-methylisoxazole (8.6 g., 73% based on the mixture) of m.p. 217~221°.[3] This compound (0.81 g.) was dissolved in 4 ml. of 10% NaOH. The mixture warmed on a water bath at 90° for 1 hr. After cooling, AcOH was added and 3-sulfanilamido-5-methylisoxazole (VIII) precipitated. Recrystallization from aq. EtOH gave white prisms of m.p. 166~167°, which showed no depression in m.p. on admixture with an authentic sample.[3] Yield, 0.67 g. *Anal.* Calcd. for $C_{10}H_{11}O_3N_3S$: C, 47.42; H, 4.38; N, 16.95. Found: C, 47.69; H, 4.35; N, 16.72.

5-Aminoisoxazole (IX)——Propiolonitrile, prepared from propiolamide (6.0 g,. 0.087 mole), P_2O_5 (48.0 g.) according to Moureu's method,[21] was dissolved in 35 ml. of MeOH and mixed with hydroxylamine hydrochloride (6.0 g., 0.087 mole) in 10% NaOH (34.8 ml., 0.087 mole). The reaction mixture was neutral to litmus. After standing for a night at 30°, the reaction mixture was saturated with NaCl and extracted three times with ether. The extract was dried over Na_2SO_4, filtered and the solvent was evaporated. Recrystallization of the residue from $CHCl_3$ afforded white needles of 5-aminoisoxazole (IX), m.p. 75~77°. Yield, 4.62 g. (63% based on propiolamide). *Anal.* Calcd. for $C_3H_4ON_2$: C, 42.85; H, 4.80; N, 33.32. Found: C, 42.52; H, 4.67; N, 33.57.

5-Benzamidoisoxazole (X)——A solution of IX (0.246 g.) in pyridine (1 ml.) was mixed with benzoyl chloride (0.410 g.) in 1 ml. of pyridine. After 30 min., the reaction mixture was diluted with 20 ml. of H_2O. Colorless oily material separated and crystallized on cooling. Recrystallization from benzene afforded colorless prisms of 5-benzamidoisoxazole (X), m.p. 134~135°. Yield, 0.370 g. (62%). UV λ_{max} mμ (ε): 229.5 (9600), 266 (15130). λ_{min} mμ (ε): 241 (8520). *Anal.* Calcd. for $C_{10}H_8O_2N_2$: C, 63.82; H, 4.29; N, 14.89. Found: C, 63.53; H, 4.22; N, 14.81.

3-Aminoisoxazole (XI)——To a mixture of hydroxylamine hydrochloride (6.0 g., 0.087 mole) and 10% NaOH (70 ml., 0.175 mole) was added a solution of propiolonitrile, prepared from propiolamide (6.0 g., 0.087 mole), in 70 ml. of EtOH. After standing overnight at 30°, the reaction mixture was saturated with NaCl and extracted with six portions of 50 ml. of ether. The combined extract was dried over Na_2SO_4, filtered and the solvent was evaporated. The oily residue was distilled to give 3-aminoisoxazole (XI) of b.p_4 75~76°. Yield, 3.4 g. (44% based on propiolamide). *Anal.* Calcd. for $C_3H_4ON_2$: C, 42.85; H, 4.80; N, 33.32. Found: C, 43.20; H, 4.91; N, 32.89.

3-Benzamidoisoxazole (XII)——To a solution of XI (0.200 g.) in 1.5 ml. of pyridine was added dropwise benzoyl chloride (0.340 g.) under ice cooling. After 6 hr.-standing, 10 ml. of H_2O was added to the mixture. White precipitate was collected, washed with H_2O and recrystallized from benzene to colorless needles of 3-benzamidoisoxazole (XII), m.p. 148~149° (lit. m.p. 149°).[1] Yield, 0.291 g. (62%). UV λ_{max} mμ (ε): 237.5 (15950). *Anal.* Calcd. for $C_{10}H_8O_2N_2$: C, 63.82; H, 4.29; N, 14.89. Found: C, 63.64; H, 4.18; N, 14.97.

3-Acetylsulfanilamidoisoxazole——To a solution of (XII) (0.95 g.) in 5 ml. of pyridine was added acetylsulfanilyl chloride (2.66 g.) under cooling. After 1 hr.-standing at room temperature, the reaction mixture was poured into 50 ml. of H_2O and dried under reduced pressure. The white powder, weighed 3.30 g., was recrystallized from EtOH to give colorless prisms of 3-acetylsulfanilamidoisoxazole, m.p. 245~247° (decomp.). Yield, 2.55 g. (80%). *Anal.* Calcd. for $C_{11}H_{11}O_4N_3S$: C, 46.96; H, 3.94; N, 14.83. Found: C, 47.02; H, 4.10; N, 15.03.

32) R. Vessiere, F. Therson: Compt. rend., **255**, 3424 (1962).

3-Sulfanilamidoisoxazole (XIII)——3-Acetylsulfanilamidoisoxazole (0.459 g.) was dissolved in 2 ml. of 10% NaOH and heated on a water bath at 80° for 1 hr. After cooling, AcOH was added to pricipitate 0.327 g. (83.5%) of 3-sulfanilamidoisoxazole (XIII). Recrystallization from 50% EtOH gave white prisms of m.p. 124~126°. *Anal.* Calcd. for $C_9H_9O_3N_3S$: C, 45.18; H, 3.79; N, 17.56. Found: C, 45.34; H, 3.91; N, 17.85.

3-Hydroxy-5-phenylisoxazole (XIV)——To a mixture of hydroxylamine hydrochloride (8.4 g., 0.12 mole) and 10% NaOH (160 ml., 0.4 mole) was added a solution of ethyl phenylpropiolate (6.96 g., 0.04 mole) in 160 ml. of EtOH. After stirred for a night at 30°, the reaction mixture was acidified with conc. HCl to pH 2. The precipitate was taken up in ether and the water layer was further extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The white residue (5.6 g., 87%) was recrystallized from EtOH or benzene to give white prisms of 3-hydroxy-5-phenylisoxazole (XIV), m.p. 163~165°, whose infrared spectrum was superimposable on that of an authentic sample.[9] *Anal.* Calcd. for $C_9H_7O_2N$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.98; H, 4.30; N, 8.80.

3-Hydroxy-5-(*p*-nitrophenyl)isoxazole (XV)——To a mixture of hydroxylamine hydrochloride (6.70 g., 0.095 mole) and 10% NaOH (100 ml., 0.25 mole) was added a solution of ethyl *p*-nitrophenylpropiolate (7.33 g., 0.033 mole) in 100 ml. of EtOH. After stirred for a night at 20~25°, the reaction mixture was acidified with conc. HCl to pH 2. The brown precipitate was taken up in ether. The ethereal layer was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from EtOH to give small needles of 3-hydroxy-5-(*p*-nitrophenyl)isoxazole (XV), m.p. 232~234°(decomp.). Yield, 5.31 g. (77%). The analytical sample of m.p. 241~244° (decomp.) was obtained by further recrystallization from dioxane. *Anal.* Calcd. for $C_9H_6O_4N_2$: C, 52.43; H, 2.93; N, 13.59. Found: C, 52.42; H, 2.98; N, 13.68.

3-Hydroxy-(*p*-chlorophenyl)isoxazole (XVI)——A solution of ethyl *p*-chlorophenylpropiolate (5.6 g., 0.0287 mole) in 50 ml. of EtOH was mixed with hydroxylamine hydrochloride (3.0 g., 0.044 mole) in 10% NaOH (48 ml., 0.12 mole). After standing overnight, the reaction mixture was diluted with H_2O, acidified with conc. HCl to pH 2 and extracted with ether. The organic layer was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from benzene to give white needles (2.7 g., 52%) of 3-hydroxy-5-(*p*-chlorophenyl)isoxazole (XVI), m.p. 220~221° (decomp.). *Anal.* Calcd. for $C_9H_6O_2NCl$: C, 55.26; H, 3.09; N, 7.16. Found: C, 55.10; H, 3.01; N, 6.96.

3-Hydroxy-5-(*p*-methoxyphenyl)isoxazole (XVII)——To a mixture of hydroxylamine hydrochloride (3.28 g., 0.0427 mole) and 10% NaOH (36 ml., 0.090 mole) was added a solution of ethyl *p*-methoxyphenylpropiolate (6.80 g., 0.0318 mole) in 30 ml. of EtOH. After stirred for a night, the reaction mixture was diluted with H_2O, acidified with conc. HCl to pH 2 and extracted three times with ether. The extract was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from benzene to give white needles (2.9 g., 48%) of 3-hydroxy-5-(*p*-methoxyphenyl)isoxazole (XVII), m.p. 212~214° (decomp.). *Anal.* Calcd. for $C_{10}H_9O_3N$: C, 62.82; H, 4.75; N, 7.33. Found: C, 62.49; H, 4.71; N, 7.22.

3-Hydroxy-5-methylisoxazole (XVIII)——To a mixture of hydroxylamine hydrochloride (13.9 g., 0.20 mole), MeOH (200 ml.) and 10% NaOH (220 ml., 0.55 mole) was added a solution of methyl tetrolate (17.0 g., 0.173 mole) in 50 ml. of MeOH. After standing overnight at 30°, the reaction mixture was acidified with conc. HCl, saturated with Na_2SO_4 and extracted three times with ether. The water layer was further extracted continuously with 300 ml. of ether for 24 hr. The combined extract was dried over Na_2SO_4 and the solvent was evaporated. The residue was extracted three times with boiling hexane. Evaporation of hexane gave 15.6 g. (92%) of crude 3-hydroxy-5-methylisoxazole (XVIII). Recrystallization from hexane gave white needles of m.p. 84~85°. *Anal.* Calcd. for $C_4H_5O_2N$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.60; H, 5.09; N, 13.89.

Tetrolohydroxamic Acids, XIX and XX——To a mixture of hydroxylamine hydrochloride (1.31 g., 0.0188 mole), 10% NaOH (15 ml., 0.0375 mole) and EtOH (10 ml.) was added a solution of methyl tetrolate (1.528 g., 0.0156 mole) in EtOH (5 ml.) at 0~2° in 30 min. After stirring at 5~10° for 30 min., the reaction mixture was acidified with conc. HCl to pH 3, saturated with Na_2SO_4 and extracted three times with ether. The organic layer was dried over Na_2SO_4, filtered and the solvent was evaporated. The residue was extracted with hot benzene. Condensation of the benzene solution gave white needles of m.p. 115~117°(XIX) (yield, 0.077 g.). *Anal.* Calcd. for $C_4H_5O_2N$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.33; H, 5.10; N, 14.14. The benzene-insoluble material was recrystallized from acetone to afford colorless prisms of m.p. 143~144° (XX) (yield, 0.478 g.). *Anal.* Calcd. for $C_4H_5O_2N$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.20; H, 4.96; N, 14.11. Recrystallization of XIX and XX caused no change in their IR spectra.

Conversion of XIX to XVIII——XIX (0.0128 g.) was dissolved in 1.0 ml. of 10% NaOH. After standing at 30° for a night, the reaction mixture was acidified with conc. HCl to pH 2, saturated with Na_2SO_4 and extracted four times with ether. The extract was dried over Na_2SO_4, filtered and the solvent was evaporated. The residue was recrystallized from hexane to afford 0.0093 g. (73%) of XVIII, m.p. 84~85°.

Conversion of XX to XVIII——From XX (0.100 g.) and 10% NaOH (5 ml.), 0.075 g. (75%) of XVIII was obtained as described above.

3-Hydroxyisoxazole (XXI)——To a mixture of hydroxylamine hydrochloride (13.9 g., 0.20 mole), 10% NaOH (230 ml., 0.56 mole) and EtOH (200 ml.) was added a solution of ethyl propiolate (14.7 g., 0.17 mole) in 50 ml. of EtOH. After standing overnight at 30°, the mixture was acidified with conc. HCl to pH 2, saturated

with NaCl and extracted seven times with ether. The extract was dried over Na_2SO_4 and the solvent was evaporated. The residue was recrystallized from hexane to give white prisms of 3-hydroxyisoxazole (XXI) of m.p. 98～99°. Yield, 7.5 g. (59%). *Anal.* Calcd. for $C_3H_3O_2N$: C, 42.36; H, 3.56; N, 16.47. Found: C, 42.42; H, 3.55; N, 16.60.

Reaction of Phenylpropiolic Acid with Hydroxylamine in the presence of Sodium Hydroxide——To a mixture of hydroxylamine hydrochloride (1.39 g., 0.02 mole) and 10% NaOH (20 ml., 0.05 mole) was added a solution of phenylpropiolic acid (1.46 g., 0.01 mole) in 20 ml. of EtOH. After standing ovenight at 30°, the mixture was acidified with conc. HCl and extracted with ether. The extract was washed with H_2O, dried over Na_2SO_4 and the solvent was evaporated. The residue, crude 3-phenyl-5-isoxazolone (XXII) (1.25 g., 78%), was recrystallized from aq. EtOH to give colorless prisms of m.p. 151～152°, which showed no depression in m.p. on admixture with an authentic sample prepared from ethyl benzoylacetate and hydroxylamine.[12] *Anal.* Calcd. for $C_9H_7O_2N$: C, 67.07; H, 4.38; N, 8.69. Found: C, 66.78; H, 4.42; N, 8.77.

The measurement of IR, UV and NMR spectra were carried out by Messrs. H. Higuchi, C. Fujimura, Misses N. Sawamoto, T. Li, Y. Nakajima and M. Shimada. Microanalyses were made by Messrs. K. Ono, H. Shimada, Misses K. Saito, N. Gonda, H. Masuda and M. Yamamuro.

Summary

β-Bromocinnamonitrile and hydroxylamine afforded 3-amino-5-phenylisoxazole in the presence of alkali, unexpectedly. So the reaction of acetylenic nitriles and esters with hydroxylamine was examined under alkaline conditions. Phenylpropiolonitrile and propiolonitrile gave 3-amino-5-phenylisoxazole and 3-aminoisoxazole, respectively. Tetrolonitrile afforded a 3:1 mixture of 3-amino-5-methyl- and 3-methyl-5-amino-isoxazole in the presence of alkali. Under neutral conditions these nitriles gave the corresponding 5-aminoisoxazoles. Similarly, 3-hydroxy-, 3-hydroxy-5-methyl- and 3-hydroxy-5-phenyl-isoxazole were obtained from the corresponding acetylenic esters. In the case of methyl tetrolate, two isomers of tetrolohydroxamic acid were isolated as the intermediate. On the other hand, free phenylpropiolic acid gave only 3-phenyl-5-isoxazolone under the same alkaline conditions.

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175. Hidetoshi Yoshimura, Kazuta Oguri, and Hisao Tsukamoto : Detection of Morphine in Urine. II.*1 An Improved Method by Thin-Layer Chromatography Utilizing Potassium Platinum Iodide as the Reagent for Both Coloration and Fluorescence.

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In the previous paper*1 of this series, the authors reported a simple and sensitive detection method for microgram quantities of morphine in urine, which consisted of three parts; a sufficient hydrolysis of conjugated morphine to free form, complete extraction of morphine with chloroform using continuous extractor, and its detection by double thin-layer chromatography. Recently Kupferberg, *et al.*[1,2] also developed a

*1 Part I. H. Yoshimura, K. Oguri, H. Tsukamoto : This Bulletin, **14**, 62 (1966).
*2 Katakasu, Fukuoka (吉村英敏, 小栗一太, 塚元久雄).
1) H. J. Kupferberg, A. Burkhalter, E. L. Way : J. Pharmacol. Exptl. Therap., **145**, 247 (1964).
2) *Idem* : J. Chromatog., **16**, 558 (1964).