UDC 547.867.4.07

105. Kiyoshi Takahashi, Masako Muraoka, and Takeo Ueda : Synthesis of N-Alkyl-4-morpholinecarboxamidine.

(*Pharmaceutical Institute, Keio-Gijuku University*[*1])

B. Melander[1] reported that 1,1-(2,2'-oxydiethyl)biguanide (ABOB) showed a protective *in vivo* effect on PR-8 strain of influenza A virus and Lee strain of influenza B virus in mice. After that, K. S. Plicher and K. F. Soikes[2] published that 1-isopropylbiguanide and 1,1-dimethylbiguanide exerted an *in ovo* effect on PR-8 strain in embryonated eggs, but not any *in vivo* effect on the virus in mice.

These findings apparently seemed to suggest that the structure of 1-substituted biguanide might contribute to the generation of antiinfluenzal activity and especially the introduction of morpholino group into biguanide could be associated with *in vivo* effect on the virus in mice.

In parallel with the above studies, our group took up biguanide, considered as one compound related to guanidine, which was confirmed by our group[3] to have an inhibitory effect on polio and measles virus in tissue culture, synthesized compounds of 1-substituted biguanide containing ABOB and 1,1-hexamethylenebiguanide[*2] and examined as to their *in vivo* effect on PR-8 strain in mice. Any of these compounds, however, were found not to have any persuasible *in vivo* effect against influenza virus in mice. In contrast to the report of B. Melander, our finding denies the importance of morpholino group and biguanide for the marked generation of antiinfluenzal activity in mice. Hereupon, the authors made an idea to survey the contribution of morpholino group to antiviral activity by introducing morpholino group into an antiviral compound, guanidine.

This report is concerned with the synthesis and antiviral effect of N-alkyl-4-morpholinecarboxamidine.

Synthesis of N-Alkyl-4-morpholinecarboxamidine

To find the synthetic method of N-alkyl-4-morpholinecarboxamidine, the guanidination of morpholine may be considered as a shorter route.

The first method is to obtain the objective compounds by reacting morpholine with alkylcyanamide, which is prepared through the reaction between cyanogen bromide and alkylamine,[4] as shown in Chart 1.

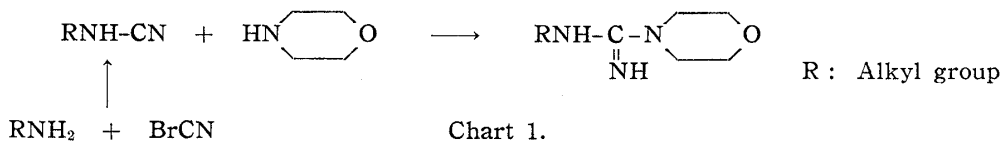


Chart 1.

This method, however, was found tremendously difficult in the isolation and purification of the objective compounds.

*1 Shinano-machi, Shinjuku-ku, Tokyo (高橋 廉, 村岡全子, 上田武雄).

*2 This compound was synthesized by our group, reacting hexamethylenimine with dicyanodiamide. Hydrochloride : Colorless prisms, m.p. 212~214°. *Anal.* Calcd. for $C_8H_{18}N_5Cl$: N, 31.88. Found: N, 31.99.

1) B. Melander : Toxicology and Applied Pharmacol., **2**, 274 (1960).
2) K.S. Plicher, K.F. Soikes : Antibiotics and Chemotherapy, **11**, 881 (1961).
3) T. Ueda, S. Toyoshima, T. Tsuji, Y. Seto : Keio J. of Med., **10**, 257 (1961); T. Ueda, S. Toyoshima, T. Tsuji, Y. Seto, J. Nomoto : Antibiotics and Chemotherapy, **12**, 330 (1962).
4) R.H. Mckee : Am. Chem. J., **36**, 210 (1906).

The second method as illustrated in Chart 2, is to synthesize the objective compounds *via* 1-alkyl-S-methylisothiourea prepared from 1-alkylthiourea and dimethyl sulfate, using alkyl isothiocyanate as the starting material.

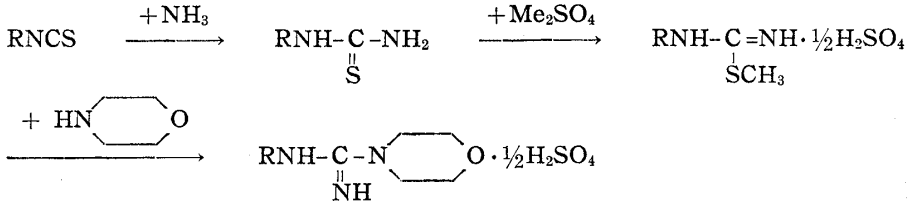


Chart 2.

In this method, the ammonolysis of alkyl isothiocyanate afforded 1-alkylthiourea and the latter could be converted to S-methylisothiourea sulfate derivative with dimethyl sulfate, from which N-alkyl-4-morpholinecarboxamidine was synthesized with difficulty by reacting with morpholine. The end-product by this method, however, was found to be difficultly purified, because it was contaminated with probably morpholine sulfate.

Thus, the third method was conceived by rearranging the synthetic order of ammonolysis, methylation and morpholination, as illustrated in Chart 3.

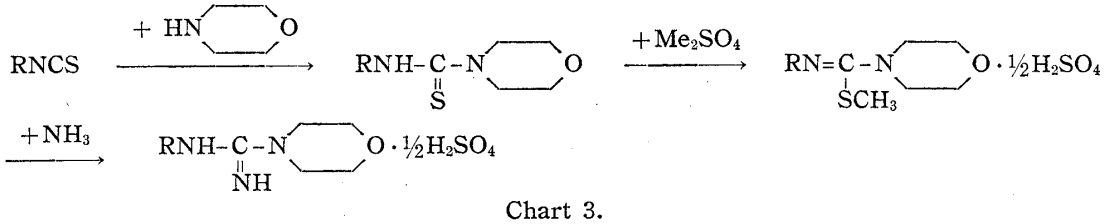


Chart 3.

This method was found suitable for the synthesis of N-alkyl-4-morpholinecarboxamidine, even if it needed the longer route than the first method.

All compounds of alkyl isothiocyanate, as the starting material, were found to be easily prepared in ca. 40~70% yield by the slight modification of the procedure reported in the synthesis of methyl isothiocyanate.[5] The compounds synthesized are listed in Table I.

Table I. Alkyl Isothiocyanate RNCS

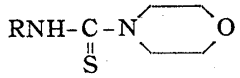
R	Formula	b.p. (°C/mm. Hg)	Yield (%)
CH_3	C_2H_3NS	119	66
C_2H_5	C_3H_5NS	131~132	60
C_3H_7	C_4H_7NS	152~153	45
C_4H_9	C_5H_9NS	88~92/48~50	48
C_5H_{11}	$C_6H_{11}NS$	98~100/32~33	40
C_6H_{13}	$C_7H_{13}NS$	102/19	51
⬡	$C_7H_{11}NS$	114~116/28~29	64

The compounds of alkyl isothiocyanate were converted to N-alkyl-4-morpholinethiocarboxamide by the reaction with morpholine. This reaction was observed to proceed with the same ease as ammonolysis of alkyl isothiocyanate to 1-alkylthiourea.[6] The compounds obtained are listed in Table II.

The compounds of N-alkyl-4-morpholinecarboxamide were converted to 1,1-(2,2'-oxydiethyl)-3-alkyl-S-methylisothiourea by the methylation with dimethyl sulfate and

5) M.L. Moore, F.S. Crossley : Org. Syntheses, **21**, 81 (1941).
6) *Idem* : *Ibid.*, **21**, 83 (1941).

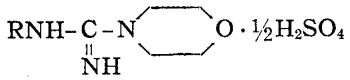
Table II. N-Alkyl-4-morpholinethiocarboxamide



R	Formula	m.p. (°C)	Analysis N (%) Calcd.	Found
CH_3	$C_6H_{12}ON_2S$	96～ 98	17.48	17.42
C_2H_5	$C_7H_{14}ON_2S$	61～ 62	16.08	16.04
C_3H_7	$C_8H_{16}ON_2S$	36～ 38	14.88	15.00
C_4H_9	$C_9H_{18}ON_2S$	46	13.85	14.01
C_5H_{11}	$C_{10}H_{20}ON_2S$	35～ 38	12.95	13.12
C_6H_{13}	$C_{11}H_{22}ON_2S$	48～ 50	12.17	12.22
⬭	$C_{11}H_{20}ON_2S$	133～135	12.27	12.23

the resulted products could be reacted by the ammonolysis to form the objective compounds. Table III shows the compounds of N-alkyl-4-morpholinecarboxamidine sulfate and their analytical data.

Table III. N-Alkyl-4-morpholinecarboxamidine Sulfate



R	Formula	Appearance	Yield (%)	Analysis N (%) Calcd.	Found
H	$C_5H_{11}ON_3 \cdot \frac{1}{2}H_2SO_4$	Colorless needles	62	23.57	23.35
CH_3	$C_6H_{13}ON_3 \cdot \frac{1}{2}H_2SO_4$	Colorless prisms	32	21.86	22.02
C_2H_5	$C_7H_{15}ON_3 \cdot \frac{1}{2}H_2SO_4$	〃	31	20.38	20.51
C_3H_7	$C_8H_{17}ON_3 \cdot \frac{1}{2}H_2SO_4$	〃	36	19.08	19.22
C_4H_9	$C_9H_{19}ON_3 \cdot \frac{1}{2}H_2SO_4$	Colorless needles	41	17.90	18.15
C_5H_{11}	$C_{10}H_{21}ON_3 \cdot \frac{1}{2}H_2SO_4$	Colorless prisms	33	16.92	17.01
C_6H_{13}	$C_{11}H_{23}ON_3 \cdot \frac{1}{2}H_2SO_4$	Colorless needles	31	16.14	16.21
⬭	$C_{11}H_{21}ON_3 \cdot \frac{1}{2}H_2SO_4$	〃	38	16.20	16.35

Screening Tests with the Compounds Synthesized

The eight compounds of N-alkyl-4-morpholinecarboxamidine were tested as to their activity on Mahoney strain of polio virus and Edmonstan strain of measles virus in tissue culture and PR-8 strain in mice.

The experimental procedures for screeing tests of polio and measles virus were the same as those described in the previous reports[3] and for the screening of anti-influenzal property the consolidation score method of Ledinko, *et al.*[7] was used.

As the experimental result obtained, any of the compounds was found inactive on any of the viruses (the experimental data are omitted).

This finding suggests that the introduction of morpholino group into the structure of guanidine inactivated the original antiviral activity of guanidine and could not contribute to the generation of antiinfluenzal activity.

In contrast of this result, our group has obtained several active compounds having morpholino and amidino groups at the both ends of a variety of heterocyclic rings. The works on this problem will be reported in the near future.

Experimental

Synthesis of N-Alkyl-4-morpholinethiocarboxamide——To 0.11 mole of morpholine was dropwise added 0.1 mole of alkyl isothiocyanate with stirring over a period of half an hour. After the addition, the stirring was continued during 2 hr. on a water bath. When the oily liquor thus obtained was chilled

7) N. Ledinko, B. Perry : J. Immunol., **74**, 371 (1955).

deeply, the thiourea began to solidify as a compact, solid mass. Purification by recrystallization from dil. MeOH yielded between 70 % and 80 % of the theoretical amount.

Synthesis of N-Alkyl-4-morpholinecarboxamidine Sulfate——A mixture of 0.1 mole of N-alkyl-4-morpholinethiocarboxamide and 6.4 g. (0.05 mole) of Me_2SO_4 was refluxed at 110° in an oil bath for 1 hr. The S-methylisothiourea sulfate was then dissolved in 20 cc. of water, and cleared with charcoal. To the filtrate was added, all at once, 20 cc. of NH_3-water. Under agitation, the solution was gently warmed on a water bath over 3 hr. After a great part of MeSH was evolved, the solution was boiled, discolored with charcoal, and then concentrated under reduced pressure until it was anhydrous. The syrupy residue was covered with a layer of dry Me_2CO or anhyd. MeOH, and chilled for several days, whereupon it set to crystallize.

Summary

The eight compounds of N-alkyl-4-morpholinecarboxamidine sulfate were synthesized *via* N-alkyl-4-morpholinethiocarboxamide from alkyl isothiocyanate, wherein alkyl group stands for H, CH_3, C_2H_5, C_3H_7, C_4H_9, C_5H_{11}, C_6H_{13}, or cyclohexyl.

Any of the compounds synthesized was found inactive on any of polio, measles and influenza virus.

(Received July 23, 1962)

UDC 547.92:577.176.3

106. Keiichi Takamura, Chifuyu Isono, Sakae Takaku, and Yoshihiro Nitta : Studies on Steroids. I. The Preparation and Properties of 17β-(N-Substituted 2-Amino-4-thiazolyl)-androst-4-en-3-one Series.

(*Research Laboratory, Chugai Pharmaceutical Co., Ltd.*[*1])

At the time of this investigation it had already been shown that modification of 17-position of androstane could give a various biological activity. Recently, Ralls and his co-workers[1] described on the synthesis of 17β-(2-substituted 4-thiazolyl)androst-4-en-3-one series possessing pharmacological activity as cardiac glucoside. On the other hand, Schaub, *et al.*[2] also reported on the synthesis of 17β-(2-amino-4-thiazolyl)androst-4-en-3-one series having no significant activity. The present authors attempted to prepare a various kinds of 17β-(N-substituted 2-amino-4-thiazolyl)androst-4-en-3-one series in order to examine the structure-activity relationships.

Synthesis of 17β-(2-alkylamino-(or 2-arylamino- or 2-N,N-alkylarylamino-)-4-thiazolyl)-androst-4-en-3-one Series

21 Mesylates (II) of Reichstein's compound S, cortisone and desoxycorticosterone, were prepared by treatment of the parent compounds with methanesulfonyl chloride in pyridine at low temperature respectively.

The conversion of II to 21-iodides derivatives (III) were carried out with use of sodium iodide in acetone. 21-Iodides (III) were condensed with thiourea, N-alkylthiourea

*1 Takadaminami-cho, Toshima-ku, Tokyo (高村圭一, 磯野千冬, 高久 栄, 新田義博).

1) J. W. Ralls, M. Grove, C. G. Bergstrom : U. S. Pat. 2,793,207 (1957).
2) R. E. Schaub, M. J. Weiss : J. Org. Chem., **26**, 1223 (1961).