CHEMISTRY LETTERS, pp. 949-952, 1972. Published by the Chemical Society of Japan

N,N'-CARBONYLDI-OXADIAZOLONE, A NEW COUPLING REAGENT FOR PEPTIDE SYNTHESIS

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A new condensing reagent, N,N'-carbonyldi-(3R-1,2,4-)oxadiazole-5-one (CDOD), is synthesized. The application to the peptide synthesis is achieved and several known peptides are prepared in good yields. The coupling reaction proceeds below 0°C over a short period. Racemization is ivestigated by the use of Anderson test.

Heterocyclic amides, such as imidazolides (CDI),[1] pyrazolides,[2] and 1,2,4-triazolides (CDT),[3] were introduced in peptide chemistry from 1958 to 1961. The studies on the more active analogous compounds than those have hardly progressed during the last ten years.

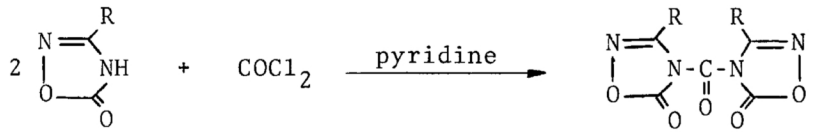
Among them, CDI and CDT are very useful reagents for the peptide coupling, but the coupling reaction by the use of both reagents takes a long period. It is interesting to introduce various substituents onto the heterocyclic compound in order to enhance the reactivity of "azolides".[3]

It may be expected that the reactivity of a reagent such as CDI would be increased by the participation of neighboring groups which decrease an electron density of the acylated imino nitrogen. Along this purpose, we have investigated the modification of the heterocyclic compounds, which are consisted of the other hetero atoms on the ring together with nitrogen atoms.

From this situation, 3R-1,2,4-oxadiazole-5-one (OD),[4] whose Pka is 5.9-6.1, is chosen as one of the compounds which are suitable for our purpose. 2R-1,3,4-derivatives, however, are found to be less adequate because of their high Pka (8.01).

We wish to report the synthesis of N,N'-carbonyldi-(3R-1,2,4-)oxadiazole-5-one (CDOD), and the successful use of this reagent in the peptide synthesis.

The mother compound of CDOD, OD has not only acidic hydrogen atom attached to heterocyclic nitrogen atom, but also carbonyl group neighboring to the same nitrogen atom. CDOD (R=phenyl) is prepared from OD (R=phenyl) and phosgene in anhydrous THF in the presence of an equivalent amount of anhydrous pyridine in 95% yield according to Beyerman *et al.*[3] Recrystallization of the crude material from chloroform and ether gives analytically pure CDOD (R=phenyl), mp 183-185°C; Found: C, 58.02; H, 2.93; N, 15.74%. Calcd for $C_{17}H_{10}N_4O_5$: C, 58.29; H, 2.88; N, 16.00%. IR (Nujol): 1810, 1785, 1775 (C=O) cm^{-1}. NMR ($CDCl_3$): 2.48 (singlet, aromatic protons). CDOD (R=benzyl) is also prepared in the same manner as above in 93% yield: mp 122-124°C; Found: C, 59.95; H, 3.71; N, 14.94%. Calcd for $C_{19}H_{14}N_4O_5$: C, 60.32; H, 3.73; N, 14.81%. IR (Nujol): 1815, 1785, 1770 (C=O) cm^{-1}. NMR ($CDCl_3$): 2.6-3.0 (multiplet, 12H, aromatic protons), 5.9 (singlet, 4H, methylene protons).



(R=phenyl or benzyl)

In regard to this reagent, the carbonyl group which lies between the heterocyclic compound is a member of so called "di-acyl type carbonyl". The reactivity of the carbonyl which takes part in the coupling reaction is so much enhanced by the neighboring carbonyl groups that coupling reaction may proceed below 0°C for more than an hour. The quantity of the crude reagent can be assayed by NMR spectrum. The reagent corresponding to 0.01 mole which was determined by the NMR was added to 0.01 mole of an acyl amino acid in anhydrous THF below -10°C and to the reaction mixture, 0.01 mole of tertiary weak base, such as N-ethylmorpholine, was added to adjust the pH of the mixture at 6 to 6.5. After the reaction mixture was allowed to stirr for 30 minutes or more at the same temperature, the desired amino acid or peptide esters in 0.01 mole quantity was added. Then the reaction was continued for 30 minutes or more at -5°C to 0°C. The solvent was distilled off under vacuum, and the residue was triturated with water and ethyl acetate and worked up to isolate the desired peptides, in the usual way.

In the case of the acyl amino acid compornent such as an acyl asparagine, glutamine, and arginine, the isolated CDOD (crude reagent) should be employed be-

cause of low solubility of the corresponding peptides in THF. In most cases, however, the THF suspension of the reagent without isolation was used for the coupling reaction.

This reagent which was isolated or non-isolated was applied to the synthesis of several known peptides shown in the Table.

In comparison with the reactivity of other "azolides" such as CDI and CDT, CDOD is so markedly reactive because of the inductive effect of the neighboring carbonyl group that the rapid formation of peptide bond is carried out. Indeed, the coupling reaction with CDOD proceeds below 0°C within an hour, while it takes a few hours or more (one or two days) at room temperature with CDI and CDT.[3,5]

This reagent may be employed as another merit for the solid phase peptide synthesis, which is under investigation, because of the high reactivity as well as the high solubility of both CDOD and OD in usual organic solvents.

The other important advantage of CDOD is that a lactam formation, which occurs in the synthesis of arginyl peptides by the conventional technics, is not observed, because the reaction proceeds for a short period in the limited acidic condition (pH 6.0-6.5).

Table. Peptide derivatives prepared using N,N'-carbonyldi-oxadiazolone

	Peptides**	Yield (%)	mp*** (°C)	$[\alpha]_D$	Lit. mp (°C)	Lit. $[\alpha]_D$
1	Z-Phe-Gly-OEt	72	107-109	-24°(c 1, DMF)	110-111	-24°(DMF)
2*	Z-Phe-Gly-OEt	85.3	107-109	-24°(c 1, DMF)	110-111	-24°(DMF)
3	Boc-Phe-Val-OMe	74.2	118-119	-11.6°(c 1, DMF)	120-122	-11°(DMF)[8]
4	Boc-Trp-Met-OMe	77.5	96-98	-24.0°(c 1, MeOH)	98-99[9]	——
5	Nps-Ala-Gly-OEt	61	100-102	-41.9°(c 1, DMF)	101-102	-42°(DMF)[10]
6*	Nps-Ile-Gly-OEt	52.8	112-113	-45.9°(c 1, DMF)	114	-46°(DMF)[11]
7*	Z-Arg(NO_2)-Met-OMe	75	136-138	-24.3°(c 1, MeOH)	137-138	-25°(MeOH)[12]
8*	Z-Gln-Gly-OMe	63.5	172-173	-16.2°(c 1, EtOH)	174.5-175.5	-16°(EtOH)[13]

* Isolated reagent was used.

** The abbreviations used conform with those tentatively proposed by the IUPAC-IBC: J. Biol. Chem., 241, 3491 (1966). The amino-acid symbols denote the L-configuration.

*** All melting points are uncorrected.

So far as Boc-, Nps-, and Z-amino acids are coupled for carboxyl component with CDOD, the corresponding dipeptides are obtained *without racemization* in reasonable yields.

When Anderson test[6] was carried out under the condition above mentioned, CDOD resulted perfect racemization *via* oxazolone mechanism. At the stage of our present investigation, however, the racemization can be minimized, when the reaction carried out at -20°C by the addition of one equivalent amount of N-hydroxysuccinimide, with which the extreme racemization is reported in the case of CDI.[7]

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(Received July 12, 1972)