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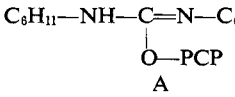
On the Optical Purity of Peptide Active Esters Prepared by N,N′-Dicyclohexylcarbodiimide and "Complexes" of N,N′-Dicyclohexylcarbodiimide–Pentachlorophenol and N,N′-Dicyclohexylcarbodiimide–Pentafluorophenol[1]

Sir:

In previous papers[2] we reported the use of active pentachlorophenyl esters for the synthesis of peptides and polypeptides. We report now the preparation of peptide-active PCPOH and PFPOH[3] esters in high optical purity and racemization studies of such esters using the Anderson[4] and Young[5] racemization tests. Active esters were prepared by (a) the backing-off procedure of Goodman,[6] (b) the "usual" method,[7] *i.e.*, from the N-protected peptides and phenol components, using N,N′-dicyclohexylcarbodiimide (DCC) as condensing agent,[8] (c) the "reverse" DCC procedure,[9] and (d) the use of a conveniently prepared crystalline "complex" consisting of the isourea derivative (A)



A

and two PCPOH. A similar structure can be assigned to the PFPOH "complex".[10] The optical purity of the peptide active esters obtained by procedures b, c, or d was determined by comparing their rotations to those of the pure compounds prepared by the backing-off procedure.[6] Remarkable is the high optical purity of the crude Z-Gly-Phe-OPCP and Z-Gly-Phe-OPFP, prepared either by the usual[11] and "reverse" methods or with the "complex," when compared with the low optical purity of the crude Z-Gly-Phe-ONP obtained by procedure b or c.[12] With the usual method at −10°, 90% optically pure Z-Gly-Phe-OPCP in 71% yield and 90 to 100% optically pure ester in 86 to 92% yield using the "complex" at room temperature[13] were obtained; similar results were obtained for the corresponding PFPOH esters. The effect of temperature and solvent has been emphasized in controlling racemization during peptide synthesis[14] and was found to be important in the preparation of active esters reported here. These conditions, however, are not sufficient to explain the substantial difference between the optical purity of the crude pentachloro-, pentafluoro-, and *p*-nitrophenyl esters. Our results indicate a parallelism between the acidity of the phenol component (p*K* value of PCPOH, 5.3; PFPOH, 5.3; and NPOH, 7.2) and the optical purity of the corresponding ester. Z-Gly-Phe-ODNP (p*K* of DNPOH, 4.1) was obtained in 98% optically pure form,[15] which fact further supports this trend. Even Bz-Leu-OH, which is more sensitive to racemization than the Anderson dipeptide,[14] was converted to a 61% optically pure PCPOH ester by the "reverse" procedure.[16]

(1) This is the seventh in a series of papers concerned with the use of pentachlorophenyl active esters.

(2) (a) J. Kovacs and A. Kapoor, *J. Am. Chem. Soc.*, **87**, 118 (1965); (b) J. Kovacs, R. Ballina, R. L. Rodin, D. Balasubramanian, and J. Applequist, *ibid.*, **87**, 119 (1965); (c) J. Kovacs and B. J. Johnson, *J. Chem. Soc.*, 6777 (1965); (d) J. Kovacs, R. Giannotti, and A. Kapoor, *J. Am. Chem. Soc.*, **88**, 2282 (1966); (e) J. Kovacs, H. N. Kovacs, J. K. Chakrabarti, and A. Kapoor, *Experientia*, **21**, 20 (1965); (f) J. Kovacs and M. Q. Ceprini, *Chem. Ind.* (London), 2100 (1965).

(3) Abbreviations used here are described in "Proceedings of the 5th European Peptide Symposium, Oxford, Sept 1962," G. T. Young, Ed., The Macmillan Co., New York, N. Y., 1963. PCPOH stands for pentachlorophenol, PFPOH for pentafluorophenol, and DNPOH for 2,4-dinitrophenol.

(4) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, **80**, 2902 (1958).

(5) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).

(6) M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).

(7) To a mixture of N-protected peptide and PCPOH, DCC was added; reactants were present in 1:1:1 molar ratio.

(8) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 77, 1067 (1955).

(9) "Reverse" DCC procedure means that DCC and 2 to 3 equiv of phenol component were allowed to stand in solution (5–30 min) before addition of the acid.

(10) These "complexes" were prepared by adding 1 mole of DDC to 3 moles of phenol component; the solvent for the DCC–PCPOH "complex" was ethyl acetate, and hexane for the DCC–PFPOH. The "complexes" deposited in crystalline form and were recrystallized from hexane; the melting point for the PCPOH "complex" is 120–165°, and 101.5–102.5° for the corresponding PFPOH "complex," with acceptable analytical values; practically no absorption at 4.7 μ in Nujol or KBr, strong band at 5.95 μ for C=N. Isourea derivatives, similar to "A," of phenols and DCC, are reported in the literature: E. Vowinkel, *Chem. Ber.*, **95**, 2997 (1962); **96**, 1702 (1963); **99**, 42 (1966); F. L. Bach, *J. Org. Chem.*, **30**, 1300 (1965). When the PCPOH "complex" was prepared in DMF, another "complex" was obtained which melted at 104–105.5°. Its analysis indicated a composition between 1 DCC and 2 PCPOH and 1 DCC and 3 PCPOH; based on its infrared spectrum, we believe it contains the above-described "complex" and some N,N′-dicyclohexyl-N-(pentachlorophenyl)urea. However, it is still usable in the preparation of active esters when used in excess; it is designated as "complex" II while the "complex" with the 1:3 ratio is designated as "complex" I. In solution, depending on the solvent, these isourea derivatives dissociate to different extents into the phenol and DCC components, as indicated by the reappearance of the 4.7-μ peak.

(11) In a recent paper D. F. De Tar, *et al.*, *J. Am. Chem. Soc.*, **88**, 1024 (1966), reported that acyl-AA-OH and Z-dipeptides gave extensively (75–100% DL) racemized pentachlorophenyl esters. We believe the differences between their results and ours, concerning the extent of racemization of PCPOH esters, are probably due to different reaction conditions.

(12) (a) The backing-off procedure, which involved the coupling of Z-Gly-OH to HBr·H-Phe-OPCP, gave after recrystallization Z-Gly-Phe-OPCP, mp 160–161°, $[\alpha]^{31}D$ −37.7° (*c* 1.03, chloroform). (b) A recrystallized sample of Z-Gly-Phe-OPFP, prepared by the backing-off procedure, melted at 96–98°, $[\alpha]^{22}D$ −9.8° (*c* 1, chloroform). (c) M. Goodman and K. C. Stueben, *J. Am. Chem. Soc.*, **81**, 3980 (1959), reported mp 146–146.5°, $[\alpha]^{25}D$ −6.5° (*c* 2.0, chloroform) for Z-Gly-Phe-ONP, prepared by the backing-off procedure. We prepared this ester by the usual procedure at −10° (in DMF–EtOAc solution, 20-hr reaction time) in 6% yield and 23% optical purity; the major product isolated was racemic Z-Gly-Phe-OH (60%). Under the same reaction conditions 71.5% Z-Gly-Phe-OPCP was obtained in 90% optical purity. By the "reverse" procedure at 0°, 57% HONP ester of 14% optical purity with correct analysis was obtained as a first crop, and from the mother liquor an additional 18% ester was isolated, which after two recrystallizations gave optically pure ester in 6% yield.

(13) Equimolecular amounts of "complex" I and Z-Gly-Phe-OH reacted in ethyl acetate. Ether was added to the reaction mixture and filtered. The crystalline material, which consisted of DCU and the active ester, was triturated with dioxane and the filtrate evaporated. The crystalline residue was washed with ether. The analyses for the crude materials were correct.

(14) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Am. Chem. Soc.*, **88**, 1338 (1966); *Acta Chim. Acad. Sci. Hung.*, **44**, 51 (1965); M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963); 3701 (1964).

(15) The usual procedure in the presence of 2 equiv of DNPOH gave, at −10°, 83% crude ester, mp 83–85°, $[\alpha]^{21}D$ −29.1° (*c* 2.01, chloroform). A recrystallized sample melted at 84–85°, $[\alpha]^{21}D$ −30.0° (*c* 2.03, chloroform) and analyzed correctly. In the Anderson test a sample with $[\alpha]D$ −29.8° proved to be optically pure.

(16) Bz-Leu-OPCP, prepared by the backing-off procedure, melted at 125–126°, $[\alpha]^{26}D$ −34.2° (*c* 1.09, chloroform).

In the case of the tripeptide, Z-Gly-Gly-Phe-OPCP, when prepared by the usual procedure, only 52% optical purity was obtained at −10°, while the "complex" gave 99% optically pure product in 85% yield at room temperature.[17]

Generally the use of the "complex" at room temperature resulted in higher yields and optical purity of esters. This raised the possibility that Z-Gly-Phe-OH makes a direct nucleophilic attack on the aromatic nucleus of "A." This reaction path was eliminated by O^{18} studies.[18] Z-Gly-OH, used as a model, containing 1.52% O^{18} in the carboxyl group, when allowed to react with the PCPOH- or PFPOH–isourea "complexes," gave DCU with 0.76% O^{18}; no labeled DCU would have resulted from a direct nucleophilic attack. Reaction of Z-Gly-Phe-OH with the PCPOH "complex," when followed by infrared spectroscopy, showed that at 8 min, *ca.* 68% oxazolone (5.47 μ),[11,19] 14% active ester (5.60 μ), and some unreacted DCC (4.72 μ) were present, and 85% DCU was isolated at the same time. This excludes anhydride and O-acylisourea as major identifiable intermediates. These results can be explained by the dissociation of the "complex" into DCC and PCPOH followed by reaction of the Z-peptide acid with DCC, forming oxazolone through the O-acylisourea.

However, the interception of the intermediate acylisourea by the phenols is not completely excluded, based on the following observations. Infrared studies showed that active ester formation is substantially faster during the reaction of Z-Gly-OH, PCPOH, and DCC than during the reaction of $(Z\text{-Gly})_2O$, PCPOH, and DCC, as base catalyst, present in 1:2:1 ratio; this indicates that the Z-Gly-OPCP active ester is formed preferably through direct attack by the PCPOH on the acylisourea intermediate.

We conclude that the more acidic PCPOH or PFPOH which is present in excess contributes to depress the base (DCC) catalyzed racemization of the oxazolone,[19] and also opens the ring faster than *p*-nitrophenol. This faster ring opening was established by infrared studies of the reaction of PCPOH and NPOH with the oxazolone of Z-Gly-Phe-OH as well as by yields of the PCPOH and NPOH esters prepared by method b under identical conditions.[12]

In the Anderson test[4] Z-Gly-Phe-OPCP in DMF or dioxane gave 89 and 94% Z-Gly-Phe-Gly-OEt, respectively, and no DL isomer was detected in either case. This also indicates that peptide PCPOH esters can form amide bonds without racemization in the same solvent (DMF) and under similar conditions used for polymerization.[2] Z-Gly-Phe-OPFP in the Anderson test, in DMF, also gave no racemate. Bz-Leu-OPCP in dioxane at room temperature or chloroform at 0° gave no racemate using Young's test,[5] while in THF at 0°, 9.5% DL-Bz-Leu-Gly-OH was obtained, indicating that unfavorable conditions can cause racemization.

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(20) Visiting Scientist from Budapest, Hungary, 1965–1966.

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Characterization of the Reactive Excited State in the Photochemistry of a Cyclohexadienone[1]

Sir:

The characterization of the reactive excited state(s) involved in the conversion of 2,5-cyclohexadienones to lumiproducts is a matter of extensive current interest. Fisch and Richards[2] observed that the santonin–lumisantonin photoconversion was sensitized by benzophenone and was totally quenched by solvent piperylene (although the expected *cis–trans* isomerization was not observed in dilute solution), strongly implicating triplet states in the rearrangement. They suggested the configuration of the reacting triplet was π,π^*. Zimmerman and Swenton[3] proposed that a triplet was involved in the conversion of 4,4-diphenylcyclohexadienone to its lumiproduct on the basis of sensitization by acetophenone, although quenching by naphthalene was not observed, and they assigned an n,π^* configuration to the reacting triplet. Phosphorescence was observed in both studies, the emission being broad and unresolved for santonin[2] and resolved into vibrational bands for 4,4-diphenylcyclohexadienone.[3] Saltiel[4] has pointed out that Zimmerman and Swenton's data suggest but do not demand that a triplet state is involved in the rearrangement. Implicit in this criticism is the possible danger of extrapolating from spectroscopic results obtained at very low temperatures to reactions at higher temperatures. We now report the results of a study which allow unambiguous characterization of a dienone excited state.

It is well established that a characteristic reaction of ketone triplet n,π^* excited states is inter- or intramolecular hydrogen abstraction leading to alcohol or pinacol.[5,6] We have shown that photochemical hydrogen abstraction accompanied by loss of a CCl_3 group to form *p*-cresol occurs on photolysis of dienone **1** in ethyl ether and isopropyl alcohol and, to a lesser extent, in hexane and cumene.[7,8] The reaction depends

(17) Z-Gly-OH was coupled to HBr·H-Gly-Phe-OPCP by the mixed anhydride procedure. This tripeptide active ester exhibits polymorphism; from ethyl acetate mp 171–172°, from chloroform–petroleum ether, mp 137–138.5°; $[\alpha]^{21}D$ −34.8° (*c* 1.00, DMF). We reported previously[2d] mp 112–113°, $[\alpha]^{25}D$ −10° (*c* 1, chloroform). Based on this $[\alpha]$ value, the previously reported[2d] material is considered to be a partially racemized product.

(18) The O^{18} analyses were carried out by Dr. James E. Morgan, Morgan and Schaffer Corp., Montreal, Canada.

(19) (a) M. Goodman and K. C. Stueben, *J. Org. Chem.*, **27**, 3409 (1962); (b) M. Goodman and L. Levine, *J. Am. Chem. Soc.*, **86**, 2918 (1964); (c) M. Goodman and W. J. McGahren, *ibid.*, **87**, 3028 (1965); (d) I. Antonovics and G. T. Young, *Chem. Commun.*, 398 (1965); (e) E. Schnabel, *Ann.*, **688**, 238 (1965).

(1) (a) The authors wish to express their appreciation to the U. S. Army Research Office (Durham) for generous support. (b) Part X of a series on the photochemistry of unsaturated ketones in solution. Part IX: D. I. Schuster and A. C. Fabian, *Tetrahedron Letters*, 4093 (1966).

(2) M. H. Fisch and J. H. Richards, *J. Am. Chem. Soc.*, **85**, 3029 (1963).

(3) H. E. Zimmerman and J. S. Swenton, *ibid.*, **86**, 1436 (1964).

(4) J. Saltiel, "Survey of Progress in Chemistry," Vol. 2, A. F. Scott, Ed., Academic Press Inc., New York, N. Y., 1964, p 299.

(5) W. M. Moore, G. S. Hammond, and R. P. Foss, Jr., *J. Am. Chem. Soc.*, **83**, 2789 (1961); G. S. Hammond, W. P. Baker, and W. M. Moore, *ibid.*, **83**, 2795 (1961).

(6) E. J. Baum, J. K. S. Wan, and J. N. Pitts, Jr., *ibid.*, **88**, 2652 (1966), and references cited therein.