New Probes for the Study of Acylation Reactions

Sir:

The action of dehydrating agents on carboxylic acids results in the formation of anhydrides. In the presence of nucleophiles acylation occurs: the acylating agent may be the anhydride, some activated intermediate from which the anhydride itself arises, or both intermediates. In peptide synthesis, particularly in solid phase peptide synthesis where the presence of anhydrides may give rise to inhomogeneous products,[1] it becomes important to distinguish between these pathways.[2] We report two complementary probes which permit this distinction to be made.

The first of these may be considered a permutation of the method of kinetic resolution and involves the use of optically active dehydrating agents. A mixture of an achiral carboxylic acid and racemic amine is exposed to a limited quantity of an optically active dehydrating agent (e.g., the carbodiimide, Scheme I). If kinetic resolution of the amine occurs, the acylating agent involved must be the initially formed intermediate rather than the anhydride. Application of this method to the carbodiimide reaction is outlined in the scheme.

The optically active carbodiimide was prepared by the action of (+)-α-phenylethylamine on urea followed by dehydration[3] (Ph_3P-CCl_4). Three reaction conditions, using this carbodiimide as the limiting reagent, were investigated.[4] (1) The carbodiimide was added to a solution of excess benzoic acid and racemic α-phenylethylamine. The resulting benzamide[5] showed no rotation, a result consistent with the intermediacy of the anhydride. (2) When the acid was added slowly to a solution of the amine and carbodiimide, kinetic resolution occurred, indicating that the intermediate *O*-benzoylisourea had been trapped.[6] (3) The carbodiimide was added to an equimolar solution of acid and amine yielding benzamide with some optical activity, thus revealing the presence of both intermediates. Using the value observed in the second case (4:1) as the selectivity of the *O*-benzoylisourea for the enantiomeric amines, 60% of the product in reaction 3 can be calculated to arise via the anhydride.

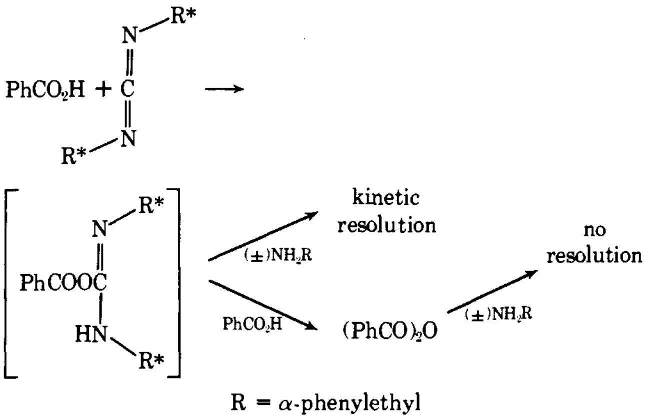
Table I. Kinetic Resolution

$$\text{acid} + (\pm)\text{-amine} \xrightarrow[25°]{CH_2Cl_2} \text{benzamide} + \text{carbodiimide}$$

	Initial concentrations (*M*)			Product rotation (deg)	% enantiomeric excess
	Acid	(±)-Amine	Carbodiimide		
1	2.4	0.8	0.08	0	0
2	<0.1[a]	2.0	0.2	−60[b]	60
3	0.8	0.8		−25	25

[a] A total of 1 equiv of acid was added as a solution to 1 equiv of amine and 0.1 equiv of carbodiimide. [b] The rotation of the pure enantiomer is −104°.

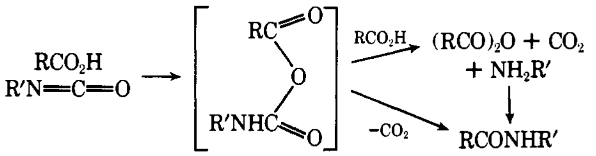
Scheme I



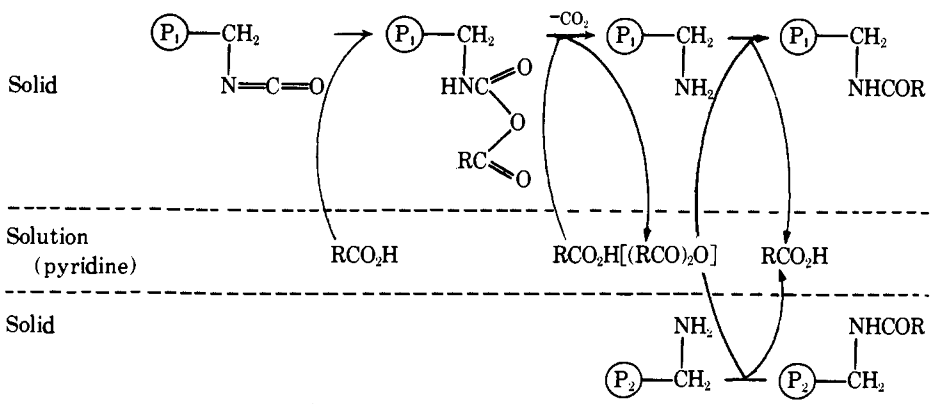
The reaction of acids with isocyanates, also useful in amide synthesis,[7] poses a related problem concerning the nature of the acylating agent (Scheme II). The product could arise from the mixed carboxylic-carbamic anhydride either by intramolecular rearrangement or by disproportionation to the symmetrical anhydride and amine.

The use of polymer-bound reagents permits the selective trapping of the symmetrical anhydride. Thus, when the polymer-bound isocyanate (Scheme III), prepared from the corresponding amine with phosgene in xylene, was treated with a solution of benzyloxycarbonyl glycine in warm pyridine, acylation occurred readily to give the polymer-bound glycine derivative. However, when the reaction was performed in the presence of a second polymer-bound reagent (the amine, P_2) 80% of the acylation occurred on this latter polymer.[8] Since the acylation of the polymer-bound amine requires the existence of an acylating agent in solution, the anhydride (or the acyl pyridinium salt)[9] must be the major immediate precursor to the amide product in the acid-isocyanate reaction. The intramolecular rearrangement of the

Scheme II



Scheme III



Ⓟ = crosslinked polystyrene
R = $C_6H_5CH_2O_2CNHCH_2$—

mixed anhydride can occur to a minor (20%) extent, if at all.

In conclusion, the use of optically active dehydrating agents permits the detection of acylating agents other than anhydrides and provides a new method for kinetic resolution. Applied to the carbodiimide reaction, the results emphasize that the actual acylating agent is very much a function of reaction conditions. Alternatively, the use of polymer-bound reagents allows the detection of anhydrides (and other reaction intermediates[10]) and shows the symmetrical anhydride to be the acylating agent in the acid-isocyanate reaction.

Acknowledgment. This work was supported by NIH Grant GM 22044.

References and Notes

(1) R. B. Merrifield, A. R. Mitchell, and J. E. Clarke, *J. Org. Chem.*, **39**, 660 (1974).

(2) J. Rebek in "Peptides, 1974", Proceedings of the 13th European Peptide Symposium, Y. Wolman, Ed., in press.

(3) Satisfactory elemental analyses and expected spectroscopic features were obtained for all new compounds. The carbodiimide derived from the (+) amine boils at 120° (bath temp) (04 mm) and shows $[\alpha]^{25}\text{D}$ −14.5° (*c* 2, acetone).

(4) The first reaction reflects conditions of solid phase peptide synthesis; the second has been recommended for solid phase synthesis in cases where diketopiperazine formation is a competing reaction (B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3012 (1972)); the final conditions are those of peptide synthesis in solution.

(5) Control experiments established that no fractionation of racemate and enantiomer occurred during the isolation of the benzamide product (distillation). The benzamide of (+)-α-phenylethylamine (L. Skulski, G. Palmer, and M. Calvin, *Rocz. Chem.*, **38**, 789 (1964)) showed $[\alpha]^{25}\text{D}$ −104° (*c* 1, DMF).

(6) The *O*-benzoylisourea reacts preferentially with the amine of the same absolute configuration.

(7) For a review see S. Goldschmidt and H. Krauss in "Newer Methods of Preparative Organic Chemistry", W. Foerst, Ed., Academic Press, New York, N.Y., 1963, p 31.

(8) Resins of differing mesh size were used and separated after reaction by screening. Direct reactions between the two solid phases have been shown to be negligible in related cases, and acyl transfer to P_2 occurs even when the two resins are separated by a glass frit.

(9) Our preference for the anhydride (rather than the acyl pyridinium species) in Scheme III is based on the observation of acyl transfer between the two resins even when toluene is used as a solvent. However, the acylpyridinium species is quite likely in the solvent shown.

(10) J. Rebek and F. Gavina, *J. Am. Chem. Soc.*, **96**, 7112 (1974); J. Rebek, D. Brown, and S. Zimmerman, *ibid.*, **97**, 454 (1975).

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The Structures of $Co_4(CO)_{12}$ and $Co_4(CO)_{11}P(OCH_3)_3$ in Solution[1]

Sir:

The recent discussion by Evans et al.[2] of the structure of $Co_4(CO)_{12}$ in solution prompts us to report data recently obtained. The question at hand is whether $Co_4(CO)_{12}$ has C_{3v} or D_{2d} molecular symmetry in solution. In the solid state the structure corresponds to idealized C_{3v} point group symmetry.[3] On the other hand, the low temperature ^{13}C NMR spectrum reported by Evans et al. consists of only three lines of roughly equal intensity, corresponding to one bridging and two terminal environments. These results are consistent only with a structure possessing D_{2d} symmetry.

We have prepared ^{13}CO enriched (~13%) $Co_4(CO)_{11}P(OCH_3)_3$,[4,5] and examined its ^{13}C NMR spectrum. The spectrum in CD_2Cl_2 at −82° is shown in Figure 1A.[6] Comparison with the previously reported ^{13}C NMR spectrum of $Co_4(CO)_{12}$[2,7] is displayed in Table I.[7]

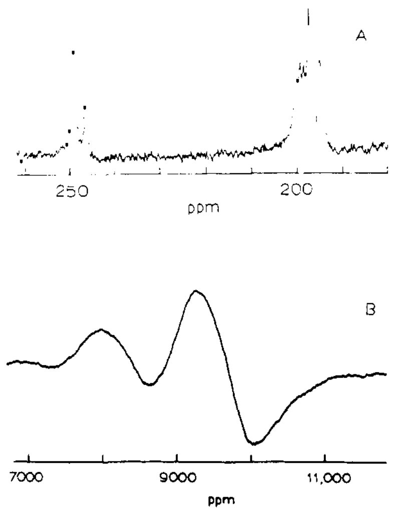

Figure 1. (A) ^{13}C FT NMR spectrum of $Co_4(CO)_{11}P(OCH_3)_3$ in CD_2Cl_2 at −82°. (B) ^{59}Co NMR spectrum of $Co_4(CO)_{12}$ in hexane at room temperature.

Table I. ^{13}C Chemical Shifts[a] in $Co_4(CO)_{11}P(OCH_3)_3$ and $Co_4(CO)_{12}$

$Co_4(CO)_{11}P(OCH_3)_3$ (−82°)			$Co_4(CO)_{12}$ (−60°)[2]
Shift	Rel int	Assignment	Shift
248.2	2	Bridging	243.1
245.6	1		
198.0 197.2	3	Basal, terminal	
196.1	3	Apical	195.9
193.7	2	Basal, terminal	191.9

[a] Downfield of TMS.

The most unequivocal result from the spectrum shown in Figure 1A is that the bridging CO resonances are in the relative intensity ratio 3:8 with respect to all the other CO's. Secondly, there are two bridging CO groups in the ratio 1:2. This strongly suggests that $Co_4(CO)_{11}P(OCH_3)_3$ is derived from the C_{3v} structure of $Co_4(CO)_{12}$, with a terminal CO group of one of the basal Co atoms replaced by $P(OCH_3)_3$, Figure 2. Furthermore, the bridging CO furthest removed from the substitution, relative intensity 1, is very close in chemical shift to the bridging CO groups of $Co_4(CO)_{12}$. Because the chemical shifts of the remaining CO groups are rather closely grouped, additional correlations between the two compounds are uncertain.

The ^{59}Co NMR spectrum of $Co_4(CO)_{12}$ has been reported twice previously.[10,11] Because of discrepancies in the reported chemical shifts we have obtained the ^{59}Co NMR spectrum at 30° in the saturated hexane solution at a higher field (~0.2 T) than previously, using a Varian WL-115 spectrometer. The spectrum is shown in Figure 1B. It is evident that there are two distinct Co resonances in roughly 1:3 intensity ratio. These occur at chemical shifts of 8400 and 9670 ppm upfield relative to $Co(NH_3)_6Cl_3$ in saturated aqueous solution as external standard.[12] These results strongly suggest that the C_{3v} form is predominant at room temperature.

An alternative possibility is that the two ^{59}Co absorptions correspond to C_{3v} and D_{2d} forms, present in roughly 1:3 ratio, respectively. This interpretation, however, is inconsistent with ir evidence. The ir spectrum of $Co_4(CO)_{12}$ in hexane at room temperature[13] shows six terminal and two bridging CO stretching absorptions. The matrix isolation spectra in Ar or N_2 at 30 K[14] are essentially identical with the room temperature solution spectra. For a C_{3v} species molecule a total of eight ir-active CO stretching modes is predicted. Finally, a KBr pellet spectrum of $Co_4(CO)_{12}$