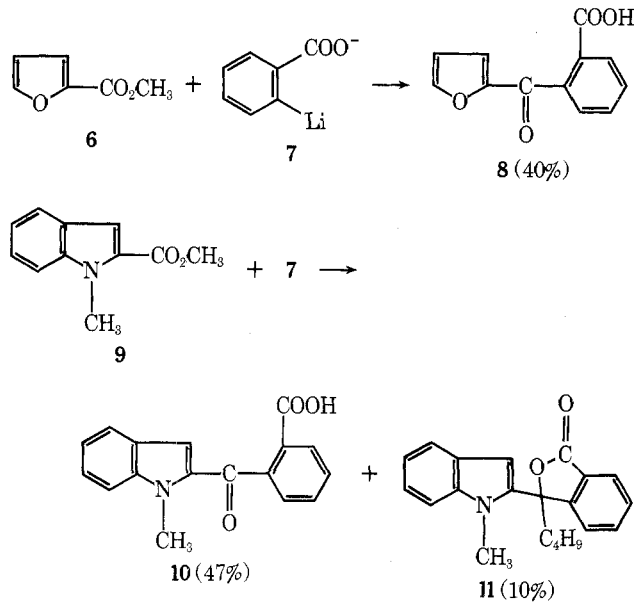
Scheme II



Incidental to this study certain heterocyclic analogues of *o*-benzoylbenzoic acid were conveniently prepared by adaptation of the method previously described[7] for benzoylbenzoic acid as shown in Scheme II.[11]

The combination of these two methods provides considerable flexibility for the synthesis of *o*-aroylbenzoic acids not easily available by other routes.

Experimental Section

General Procedure. Aryllithium derivatives **2** were prepared from the corresponding aryl halides **1** (0.02 mol) in tetrahydrofuran (80 ml distilled from $LiAlH_4$) with *n*-butyllithium (9 ml of 2.3 M solution in hexane, 0.02 mol) at −100 °C as previously described[6,7] and were added (pumped by nitrogen pressure) as rapidly as possible to a solution of phthalic anhydride (0.04 mol) in 125 ml of dry tetrahydrofuran at −100 °C. The mixture was maintained at −100 °C for 1 h and then allowed to warm to room temperature. Tetrahydrofuran was removed (in vacuo) and the solid residue shaken with a mixture of ether (60 ml) and water (100 ml). The aqueous solution was made acidic with hydrochloric acid and was extracted with ether. The ether was extracted with saturated sodium bicarbonate to remove acid. Phthalides **5** were obtained from the ether layer. The alkaline extract was acidified and the solid was collected and recrystallized as described in Table I.

2-(2-Furoyl)benzoic acid (8) was prepared from lithium *o*-lithiobenzoate (from 0.05 mol of *o*-bromobenzoic acid) as described[6] and 2-methylfuroate. After warming the mixture to room temperature, the tetrahydrofuran was removed in vacuo and water (250 ml) was added to the residue. The aqueous solution was washed with ether, acidified with hydrochloric acid, and extracted with ether (3 × 30 ml). The dried ($MgSO_4$) ether extracts were evaporated in vacuo to give the crude acid as an oil which crystallized when treated with ethyl acetate followed by evaporation of all solvent (5.5 g, 52% yield, mp 150–153 °C, mp 154–156 °C from ethyl acetate); $\nu C{=}O$ 1660, 1700 cm^{-1}.

Anal. Calcd for $C_{12}H_8O_4$: C, 66.67; H, 3.73. Found: C, 66.50; H, 3.95.

2-(1-Methyl-2-indoloyl)benzoic acid (10) was prepared from methyl 1-methylindole-2-carboxylate (0.03 mol) essentially as described for **8**. Tetrahydrofuran was removed from the crude reaction mixture in vacuo and water (250 ml) was added to the residue. The resulting mixture was extracted with ether. From the ether extract there was obtained 0.38 g (7%) of starting ester. The mixture of acids obtained by acidification of the alkaline layer was collected (ether extraction) and recrystallized from benzene to give 4.00 g (47% yield) of pure 2-(1-methyl-2-indoloyl)benzoic acid (**10**), mp 164–165 °C.

Anal. Calcd for $C_{17}H_{13}NO_3$: C, 73.10; H, 4.69; N, 5.01. Found: C, 72.97; H, 4.62; N, 4.91.

Evaporation of the benzene from which **10** was crystallized gave a semisolid to which some cold ether was added. The resulting solid was collected and recrystallized from ethyl acetate to give 0.88 g (mp 131–132 °C 10% yield) of lactone **11**.

Anal. Calcd for $C_{21}H_{21}NO_2$: C, 78.96; H, 6.62; N, 4.38. Found: C, 78.93; H, 6.50; N, 4.34.

Registry No.—**1b**, 578-57-4; **1c**, 2042-37-7; **1d**, 6952-59-6; **1e**, 623-00-7; **1f**, 577-19-5; **4b**, 1151-04-8; **4c**, 57901-51-6; **4d**, 57901-52-7; **4e**, 20643-60-1; **4f**, 57901-53-8; **6**, 611-13-2; **8**, 57901-54-9; **9**, 37493-34-8; **10**, 57901-55-0; **11**, 57901-56-1; phthalic anhydride, 85-44-9; lithium *o*-lithiobenzoate, 57901-57-2.

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(1) Supported by Grant DAHCO4-74-GD128 by U.S. Army Research Office.
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Side Reactions in Peptide Synthesis. III.[1] Intermolecular Acylation by an Unprotected Side Chain Carboxyl Group[2]

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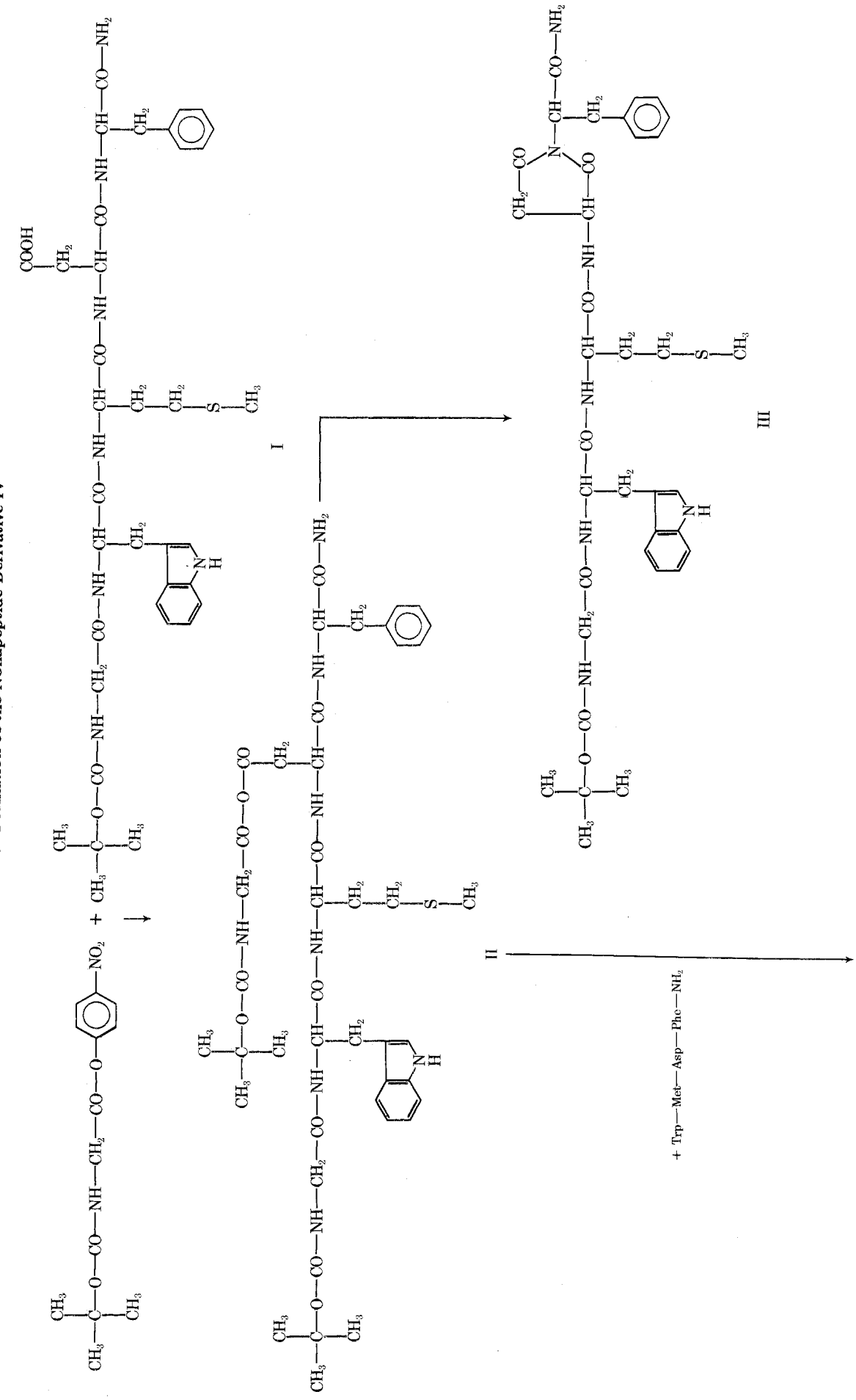
In a recent communication[1] we reported a side reaction in the preparation of the pentapeptide derivative *tert*-butyloxycarbonylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalaninamide (I, Chart I), an intermediate in the syntheses of gastrin,[3] cholecystokinin[1], and caerulein.[4] The conditions of the reaction suggested the mixed anhydride II as the reactive intermediate leading—through *intramolecular* acylation—to the by-product III, a succinimide derivative. Our conclusion that the unprotected carboxyl of the aspartyl residue can compete with the amino group in the nucleophilic attack on an active ester, particularly when the latter is present in excess, is now further supported by the isolation of a second by-product.

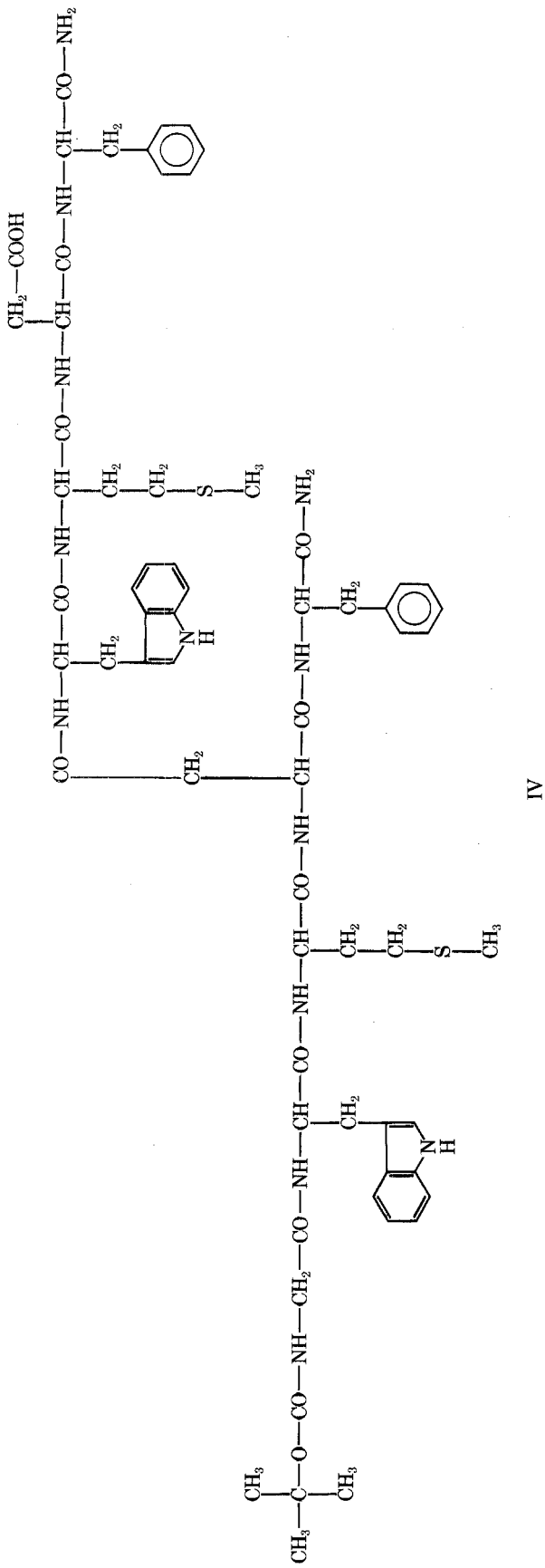
During recrystallization of samples of compound I from 95% ethanol, small amounts of a crystalline material, insoluble even in the hot solvent, were obtained. Amino acid analysis of this new by-product revealed the amino acid constituents of I, but in the molar ratio Asp 2, Gly 1, Met 2, Phe 2, Trp 2. This immediately suggested that the nonapeptide derivative IV was in hand, formed via the same mixed anhydride (II)[5] but in an *intermolecular* reaction with the amino component, a tetrapeptide amide (as shown in Chart I).

The structure proposed for compound IV was supported by its degradation (after deblocking) with cyanogen bromide[6] and with aminopeptidase M[7] (Chart II).

The unexpected[8] formation of a nonapeptide derivative (IV) during the preparation of a blocked pentapeptide (I) suggests that the protection of carboxyl groups has to be

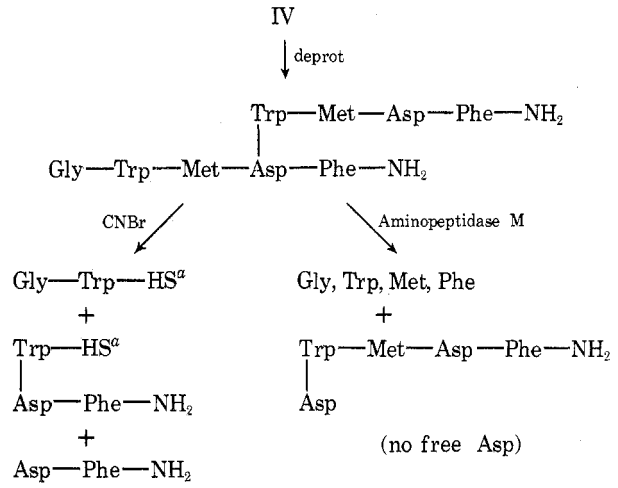
Chart I. Formation of the Nonapeptide Derivative IV





IV

Chart II. Degradation of the Nonapeptide IV



[a] HS designates both homoserine and its lactone.

considered in reactions that involve acylation with active esters.

Experimental Section

On thin layer plates of silica gel, peptides were detected by Ehrlich reagent, ninhydrin spray, and by uv. For development, the solvent system ethyl acetate–pyridine–acetic acid–water (60:20:6:11) was applied. Reagent-grade solvents were used. Amino acid analyses were carried out by the Spackman–Stein–Moore method[9] after hydrolysis with 6 N HCl at 110 °C for 16 h, unless otherwise stated.

Isolation of Compound IV. A suspension of crude protected pentapeptide amide[1] (I, 0.61 g) in 95% ethanol (15 ml) was heated on a steam bath. A small portion of the material remained undissolved and was collected by centrifugation. The solid was extracted three times with hot 95% ethanol (20 ml each). It was dissolved in dimethyl sulfoxide (1.5 ml), the solution centrifuged to remove mechanical impurities, and the solvent removed in vacuo. The residue was triturated with 95% ethanol (1 ml), centrifuged, and dried. The purified material IV (15 mg) had mp 235–237 °C, R_f 0.37. Amino acid analysis: Asp, 2.0; Gly, 1.0; Met, 2.1; Phe, 2.0; Trp (from uv), 1.9. The product gave positive reaction with Ehrlich reagent and negative reaction with ninhydrin.

Degradation of (Deblocked) Compound IV with Cyanogen Bromide. Compound IV (12 mg) was dissolved in 70% formic acid (1 ml) and kept at room temperature for 3 h. The solution was evaporated to dryness in vacuo and the deprotected peptide dissolved in 70% formic acid (1.5 ml). Cyanogen bromide (150 mg) was added and the solution stirred at room temperature for 20 h. Water (8.5 ml) was added and the solution was lyophilized. A preparative TLC on silica gel (20 × 20 cm plate) was carried out; the plate was developed four times. The bands were located by uv fluorescence. The major bands were eluted with the solvent system used for TLC (5 ml) and then with ethanol (95%, 5 ml). The solutions were concentrated and hydrolyzed for amino acid analysis. Band I (R_f 0.47): Asp, 0.8; homoserine, 0.5; Phe, 1.0; homoserine lactone, 0.4; Trp (mostly dec), trace. Band II (R_f 0.42): homoserine, 0.4; Gly, 1.0; homoserine lactone, 0.4; Trp (mostly dec), 0.1. Band III (R_f 0.19): Asp, 1.0; Phe, 0.9.

Hydrolysis of Deblocked IV with Aminopeptidase M. Compound IV (1.5 mg) was deblocked with trifluoroacetic acid (0.1 ml containing 5% anisole) at 0 °C for 30 min. The acid was removed and the residue triturated with ether. To the solid product was added 0.1 M Tris–HCl buffer (pH 7.7, 1 ml), followed by aminopeptidase M (Röhm, ca. 1000 milliunits). Toluene (4 drops) was added and the solution was incubated at 37–38 °C for 72 h. The reaction was arrested by heating the solution on a steam bath for 5 min. The solution was evaporated with a stream of nitrogen and citrate buffer (pH 2.2, 5 ml) was added. The soluble portion was separated by centrifugation and subjected to amino acid analysis: Gly, 1.0; Met, 1.0; Phe, 1.0; Trp, 0.9.

Registry No.—I, 5915-71-9; IV, 57821-06-4.

References and Notes

(1) For the previous paper in this series, cf. M. Bodanszky and S. Natarajan, *J. Org. Chem.*, **40**, 2495 (1975).
(2) This study was supported by a grant from the U.S. Public Health Service (NIH AM-12473).
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Reaction of Phosphoranes with Formate Esters. A New Method for Synthesis of Vinyl Ethers[1]

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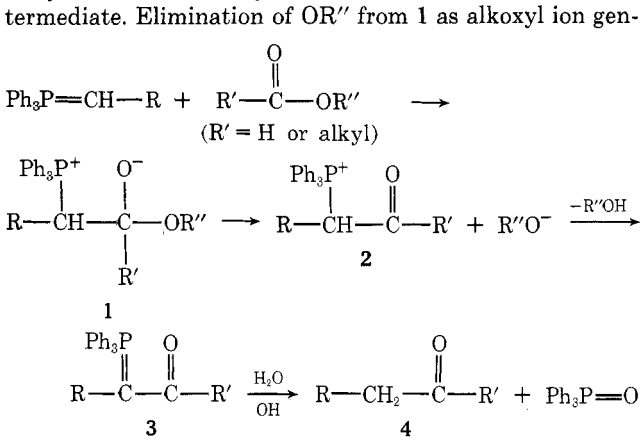
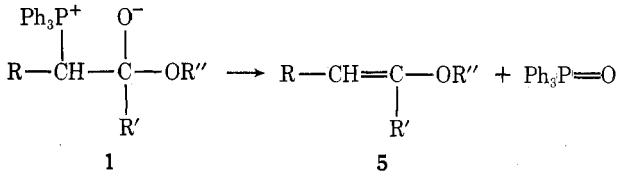
The Wittig reaction between phosphorus ylides and esters of carboxylic acids initially give β-ketoalkylphosphonium salts (**2**), which eliminate R″OH to form the stabilized β-ketoalkylidene phosphoranes (**3**).[2,4] Hydrolytic cleavage of these provides a useful synthesis of corresponding carbonyl compounds (**4**). Ethyl formate is reported to give aldehydes.[3] A reinvestigation of the reaction with formate esters was undertaken with the hope of developing a general method for the synthesis of substituted vinyl ethers instead of the reported aldehydes.[3] This study demonstrates the general feasibility for preparing vinyl ethers by this sequence.

Earlier investigations[2-5] of the mechanism of these acylation reactions indicate an initial nucleophilic attack by the ylide on the carbonyl function to form a betaine (**1**) intermediate. Elimination of OR″ from **1** as alkoxyl ion generates the phosphonium salt **2**. If such elimination is precluded, an alternative course of reaction is elimination of $Ph_3P{=}O$ to form vinyl ether **5**.

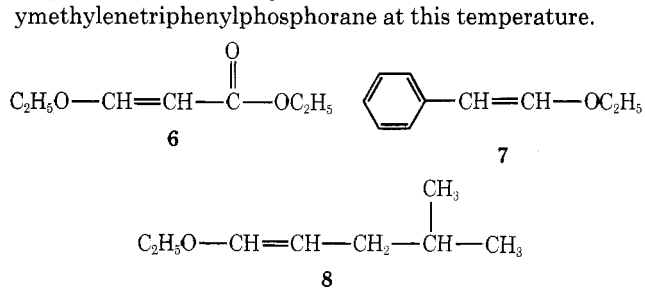




The latter possibility prompted us to explore the role, if any, of the group R′ of the ester function in determining the course of reaction. The reactions were carried out with phosphoranes ($Ph_3P{=}CHR$) that were stabilized (R = CO_2Et), partially stabilized (R = Ph), or reactive (R = alkyl or OCH_3). The esters used were those of formic, acetic, butyric, and benzoic acids and primary, secondary, and tertiary alcohols.

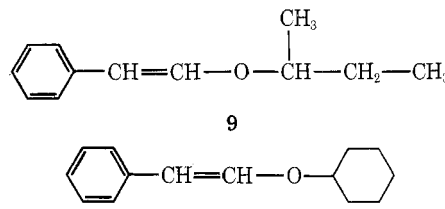
In reactions of phosphoranes with formate esters, where R′ is hydrogen, elimination of $Ph_3P{=}O$ occurred with the formation of vinyl ethers. Reaction conditions and yields depended upon the reactivity of the ylides.

The stable ylide carbethoxymethylenetriphenylphosphorane on refluxing with ethyl formate gave the vinyl ether **6** in 95% yield. A partially stabilized ylide derived from benzyltriphenylphosphonium chloride reacts with ethyl formate at room temperature to give **7** in 90% yield. However, reactive phosphoranes derived from 3-methylbutyltriphenylphosphonium bromide and methoxymethyltriphenylphosphonium chloride react with ethyl formate at room temperature to yield the reported β-formylalkylidene phosphorane.[4] When these reactions were carried out at −78 °C, the 3-methylbutylidenephosphorane gave 22% yield of vinyl ether **8**, but no vinyl ether was obtained from methoxymethylenetriphenylphosphorane at this temperature.



It appears that in reactive phosphoranes elimination of alkoxyl ion is favored at room temperature. This tendency is reduced at −78 °C and as a result some vinyl ether is formed. In the case of methoxymethylenetriphenylphosphorane, elimination of alkoxyl ion seems to be the only preferred mode of reaction, even at −78 °C. Consequently, no vinyl diether is formed.

In a second set of experiments, the partially stabilized ylide benzylidenetriphenylphosphorane was allowed to react with formate esters of *sec*-butyl alcohol and cyclohexanol. The red color characteristic of the phosphorane still remained after 8 h at 50 °C, but on work-up the corresponding vinyl ethers **9** and **10** were obtained in yields of 24



and 27%, respectively. A fourfold excess of formate esters and longer reaction times did not improve the yield. When