pound, m.p. 105–113°. This substituted oxazole when recrystallized from hot dilute alcohol was obtained as a white flaky compound which melted at 120–120.5° after drying at room temperature *in vacuo* for 2 hours.

Anal.[3] Calcd. for $C_8H_5N_2OF_3$: C, 47.53; H, 2.49; N, 13.86. Found: C, 47.80, 47.80; H, 2.76, 2.53; N, 13.16, 13.35.

DEPARTMENT OF CHEMISTRY
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RECEIVED JULY 20, 1950

Some *dl*-Alanyl-1-tyrosine Derivatives

N-Carbobenzoxy-*dl*-alanyl-1-tyrosine Ethyl Ester.—Two grams of N-carbobenzoxy-*dl*-alanine was suspended in 20 ml. of dry ether in a two-neck flask fitted with a mercury-seal stirrer and a calcium chloride drying tube. To the chilled suspension was added with stirring, 2.1 g. of phosphorus pentachloride. After one-half hour the solution was filtered into an ice-cold solution of 1.99 g. of *l*-tyrosine ethyl ester in 15 ml. of ethyl acetate. Saturated potassium bicarbonate, 20 ml., was added. After stirring for one-half hour in the cold and one hour at room temperature, the solution was transferred to a separatory funnel and was extracted with 1 *N* hydrochloric acid, half-saturated potassium bicarbonate and aqueous sodium chloride. The ether layer was dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The residue was dissolved in ethyl acetate and petroleum ether was added to an incipient turbidity. On cooling to −30° a precipitate was obtained; it was filtered and placed in a desiccator overnight. The product was dissolved in warm absolute methanol and ether was added until the solution was turbid. It was placed in the ice-box; crystallization occurred overnight. Recrystallization was effected by dissolving the crystals in a minimum amount of absolute methanol and adding petroleum ether. The product (930 mg.) melted at 132–134°.

Anal. Calcd. for $C_{22}H_{26}O_6N_2$: C, 63.75; H, 6.32. Found: C, 63.42; H, 6.51.

N-Carbobenzoxy-*dl*-alanyl-1-tyrosineamide.—One gram of the ester was dissolved in 20 ml. of absolute methanol previously saturated with dry ammonia. After standing for one week in the refrigerator, the solvent was removed under reduced pressure. The residual oil crystallized on standing in a desiccator over sulfuric acid. The amide, recrystallized from aqueous methanol, 300 mg., melted at 208–209° with decomposition.

Anal. Calcd. for $C_{20}H_{23}O_5N_3$: C, 62.32; H, 6.01; N, 11.18. Found: C, 62.42; H, 6.58; N, 10.90.

N-Carbobenzoxy-*dl*-alanyl-1-tyrosinehydrazide.—To 100 mg. of the ester, dissolved in about 1.0 ml. of absolute methanol there was added ten drops of 100% hydrazine hydrate. The solution, after standing for two hours, became a crystalline mass. The precipitate was filtered, washed with ice-cold methanol and dried. The yield, 60 mg., melted at 214–215°. On recrystallization from absolute ethanol the melting point rose to 216–217°.

Anal. Calcd. for $C_{20}H_{24}O_5N_4$: C, 59.99; H, 6.04; N, 14.01. Found: C, 59.57; H, 6.21; N, 13.46.

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RECEIVED JULY 21, 1950

COMMUNICATIONS TO THE EDITOR

DIETHYL CHLOROPHOSPHITE AS A REAGENT FOR PEPTIDE SYNTHESES

Sir:

The general methods of peptide synthesis in use involve lengthening the chain by the reaction of carboxy derivatives (halides, azides, anhydrides) with an amino group. We have found diethyl chlorophosphite to be a unique reagent in forming both reactive amides and anhydrides, thus allowing addition of aminoacid units to either end of a peptide chain.

The amides, $(C_2H_5O)_2PNHCH(R)COOR'$, are oils, at least some of which are distillable. They react with carbobenzoxyaminoacids in inert solvents to form carbobenzoxypeptide esters. The anhydrides, $(C_2H_5O)_2POCOCH(R)NHR'$, prepared in inert anhydrous solvents, are conveniently treated *in situ* with aminoacid esters. In both reactions, the by-product is presumably diethylphosphite.

Diethyl DL-α-carbethoxy-β-phenylethylaminophosphite was obtained by the reaction of DL-phenylalanine ethyl ester hydrochloride with diethyl chlorophosphite[1] and two equivalents of triethylamine in absolute ether, filtering the triethylamine hydrochloride and distilling; yield 48%, b.p. 148–151° at 0.25 mm., $n^{25}D$ 1.4908, d^{25} 1.071 (*Anal.* Calcd. for $C_{15}H_{24}NO_4P$: P, 9.88. Found: P, 9.86, 9.59.) Refluxing in toluene with carbobenzoxyglycine for one-half to two hours gave yields of 58 to 65% of carbobenzoxyglycyl-DL-phenylalanine ethyl ester[2] after recrystallization from ethanol–water, m.p. 88–90°. The phosphite derivative of glycylglycine ester reacted as an undistilled oil with carbobenzoxyglycine to give carbobenzoxydiglycylglycine ethyl ester,[3] 34% yield after recrystallization, m.p. 166–167°.

Carbobenzoxyglycine anilide (m.p. 147–148°) and carbobenzoxy-L-phenylalanine anilide (m.p. 170°), $[\alpha]^{24}D$ − 5.4° (*c*, 3; chloroform)[4] were made in good yields by the reaction of diethyl anilinophosphite[1] with the acids.

Phosphate anhydrides of carbobenzoxy- and phthalylglycine have been used for peptide syntheses.[5,6] We have found that the anhydrides of diethyl phosphite are more conveniently prepared. These are readily obtained by the reaction of diethyl chlorophosphite with carbobenzoxy- or phthalylaminoacids or peptides and triethylamine in solvents such as toluene or dioxane. Following filtration of triethylamine hydrochloride, the anhydride is treated in solution with an equivalent of an aminoacid ester at reflux for one to two hours;

(1) H. G. Cook, *et al.*, *J. Chem. Soc.*, 2921 (1949).

(2) H. Neurath, *et al.*, *J. Biol. Chem.*, **170**, 222 (1947).

(3) J. S. Fruton, *et al.*, *ibid.*, **173**, 467 (1948).

(4) The possibility of partial racemization is being investigated.

(5) H. Chantrenne, *Biochim. et Biophys. Acta*, **4**, 484 (1950).

(6) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **72**, 1312 (1950).

after concentration, the peptide derivative is washed with water and recrystallized. Dicarbobenzoxy-L-lysylglycine ethyl ester,[7] (92% yield), $[\alpha]^{25}D$ — 12.1° (*c*, 5; ethanol),[4] m.p. 90–91°, phthalylglycycl-DL-alanyl-DL-phenylalanine ethyl ester (71% yield), m.p. 189–192°, (*Anal.* Calcd. for $C_{24}H_{25}N_3O_6$: N, 9.31. Found: N, 9.56, 9.30) and carbobenzoxy, glycyl-DL-phenylalanine ethyl ester,[2] m.p. 89–90°, were prepared.

Thus by the use of one reagent units can be added to either a carboxy or amino group of a peptide derivative.

(7) M. Bergmann, *et al.*, *Z. physiol. Chem.*, **224**, 26 (1934).

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RECEIVED DECEMBER 8, 1950

THE NECESSITY FOR ACTIVATORS IN THE BORON TRIFLUORIDE CATALYZED ALKYLATION OF BENZENE BY S-BUTYL METHYL ETHER

Sir:

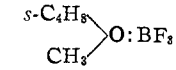
Attention has been directed recently to the necessity for the presence of traces of water or other activators in reactions catalyzed by Friedel–Crafts catalysts of the halide type. In the polymerization of ethylene,[1] isobutylene[2] and of diisobutylene[3] by boron trifluoride, traces of water are required. No suspicion of such necessity has existed where compounds known to coördinate boron trifluoride were involved.

We have found such a situation in the boron trifluoride catalyzed alkylation of benzene by *s*-butyl methyl ether. O'Connor and Sowa[4] reported that di-isopropyl ether reacts vigorously at room temperatures.

If a solution of *s*-butyl methyl ether and boron trifluoride in benzene (mole proportions 1:1:7) is prepared in apparatus exposed to the atmosphere, reaction (as noted by separation into two layers) occurs within a few days. If apparatus and materials are dried and atmospheric moisture excluded, no reaction occurs within three weeks.

Addition of water to this relatively anhydrous reaction mixture results in reaction. The added material forms a lower layer from which *schlieren* can be seen to rise until turbidity followed by large increase in the lower layer supervenes. Water reaches maximum effectiveness as an activator at a quantity equal to about 1 mole % of the boron trifluoride. Sulfuric acid and chlorosulfonic acid in similar quantities form lower layers and are even more effective than water. Methanol and acetic acid form no separate phase and are ineffective but ethanesulfonic acid, though it forms no separate phase, serves as an activator. No toluene is found in the reaction mixture; only the mono- and poly-*s*-butylbenzenes.

Boron trifluoride undoubtedly coördinates with the ether to



This complex, of itself, must be deemed relatively stable at room temperatures and not, as has been assumed, to decompose to a carbonium ion.[5] The additional intervention of a strong proton acid such as boron trifluoride monohydrate must be necessary.

In view of this behavior of secondary ethers, it is now questionable whether alkylation by analogous alcohols and esters can proceed by the process usually accepted[5]

$$RHO{:}BF_3 \longrightarrow R^+ + HOBF_3^- \qquad (1)$$

Alkylation by olefins must be similarly suspect.

(5) C. C. Price and J. M. Ciskowski, *ibid.*, **60**, 2499 (1938).

DEPARTMENT OF CHEMISTRY ROBERT L. BURWELL, JR.
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RECEIVED DECEMBER 11, 1950

GROWTH RETARDING EFFECT OF SUBSTITUTED MALONONITRILES ON TRANSPLANT TUMORS IN MICE

Sir:

The report of Hyden and Hartelius[1] that administration of malononitrile specifically produced considerable increase of polynucleotides and of protein in the nerve cells, both in experimental animals and in human beings, led the authors to undertake a program of synthesis and testing of the effect of substituted malononitriles on transplant tumors in mice. It was hoped that substitution at the active carbon of the malononitrile would lead to a more widespread effect. This indeed was found to be the case and a preliminary report of the work was presented last April at the meeting of the American Association for Cancer Research.[2]

Nearly fifty substituted malononitriles have been prepared in our laboratory (some of them not yet recorded in the literature) which according to their structure were classified into four groups as follows: (A) R-CH-$(CH(CN)_2)_2$, (B) R-CH=$C(CN)_2$, (C) R_2-C=$C(CN)_2$, (D) R-N=N-$CH(CN)_2$. The compounds were tested in strain A, C3H and C57 mice bearing the following tumors: LCS-A, S-37, C3H-S, 6C3H-ED, Eo771 and myeloid leukemia C1498, respectively. Compounds of groups A and D produced no effect in which respect they were similar to the parent compound malononitrile and to sodium cyanide, which was also tested on the tumors. Twenty compounds of group B that were screened showed an effect of varying degree from no activity to 70% retardation of growth.

To this date the two most potent compounds were the *p*-nitrobenzalmalononitrile and the 5-nitrofuranalmalononitrile. These compounds were found to be very toxic, but 200 γ and 100 γ of each in 0.1 ml. of sesame oil in daily intraperitoneal injections were tolerated by the animals (average weight 25 g.). Treatment was initiated seven days after transplantation of the tumors and

(1) F. Hofmann, *Chem. Ztg.*, **57**, 5 (1933).

(2) A. G. Evans, G. W. Meadows and M. Polanyi, *Nature*, **158**, 94 (1946); Evans and Meadows, *J. Polymer Sci.*, **4**, 359 (1949).

(3) A. G. Evans and M. A. Weinberger, *Nature*, **159**, 437 (1947).

(4) M. J. O'Connor and F. J. Sowa, THIS JOURNAL, **60**, 125 (1938).

(1) Hyden and Hartelius, *Acta Psychiatrica et Neurologica*, Suppl. XLVIII (1948).

(2) Greenberg, Irish and Gal, *Cancer Res.* (Scientific Proceedings), **10**, 221 (1950).