Phenyl-thio-carbonyl Chloride as a Group-protecting Agent

THE use of the carbobenzoxy reagent for protection of amino-groups in the course of peptide synthesis has, in spite of its obvious advantages, some limitations. In some cases, hydrogenolysis of the benzyl group is complicated by simultaneous hydrogenation of other groups present, and also carbobenzoxy compounds are sometimes difficult to crystallize; for example, *N*-carbobenzoxy-glycyl-cysteine ethyl ester. The use of another protecting unit easily removed by hydrolysis in combination with the carbobenzoxy method would considerably extend the use of the latter.

Phenyl-thio-carbonyl chloride, $C_6H_5S-COCl$, as prepared by Rivier[1], would appear to be of use in this respect. The corresponding amide and phenylamide were reported by Rivier to be stable in dilute acids and easily hydrolysed by alkalis. The extension of the method to other amides and to the amino-acid series has now been developed. Direct Schotten–Baumann acylation in aqueous alkali solution was not very successful, but the reaction of one mole of phenyl-thio-carbonyl chloride with 2 moles of amino-acid ester in ether or ethyl acetate proceeds easily at room temperature, one mole of amino-acid ester hydrochloride being liberated. After washing with dilute hydrochloric acid, the phenyl-thio-carbonyl amino-acid esters are easily isolated. Among the compounds prepared are phenyl-thio-carbonyl-glycine ethyl ester, m.p. 104°, and bis-(phenyl-thio-carbonyl)-cystine dimethyl ester, m.p. 108°.

The acylamino-acid esters thus prepared are easily de-esterified by heating for ten minutes in a mixture of concentrated hydrochloric acid and glacial acetic acid (1 : 1), the phenyl-thio-carbonyl group being remarkably stable in acids. The free acylamino-acids separate on dilution with 2 moles of water (for example, phenyl-thio-carbonyl-glycine, m.p. 155°), and can be converted to the corresponding chlorides by the action of phosphorus pentachloride in ether. The chlorides (for example, phenyl-thio-carbonyl-glycine chloride, m.p. 87°) crystallize readily and are rather stable. They react in ether or chloroform solution with free amino-acid esters to form peptide esters: phenyl-thio-carbonyl-glycine-glycine ethyl ester, m.p. 127°, phenyl-thio-carbonyl-glycine-*dl*-alanine methyl ester, m.p. 116°, phenyl-thio-carbonyl-glycine cysteine ethyl ester, m.p. 114°. The latter crystallizes readily, unlike the corresponding carbobenzoxy compound. In general, the melting points of the phenyl-thio-carbonyl derivatives lie about 50° above those of the carbobenzoxy compound.

The removal of the phenyl-thio-carbonyl-group is easily carried out by heating for five minutes with a solution of lead acetate in 70 per cent ethanol or by treating in the cold with 0·1 *N* alkali in the presence of lead hydroxide or lead carbonate, yellow lead phenylmercaptide being precipitated. In alkali, phenyl-thio-carbonyl-amino-acids are decomposed in 10–30 sec., the corresponding esters reacting similarly in 5–10 min.

This observation, together with the fact that PhS—CO—O— and PhS—CO—S compounds could be similarly prepared and split, provides a basis for an extension of peptide synthesis. Compounds of the type PhS—CO—O*R* can be prepared by heating the appropriate alcohol with phenyl-thio-carbonyl chloride either alone or with alkali (10 per cent). The alcohol can also be employed in the form of alkoxide. Phenols and SH-compounds react in the same way. The use of phenyl-thio-carbonyl chloride is capable of a number of variations and should find useful applications in other fields.

Full details of this work in connexion with peptide synthesis will be published elsewhere.

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[1] Rivier, *Bull. Soc. Chim.*, (4), **1**, 733 (1907).

Use of Seeds in the Insect Transmission of Some Plant Viruses

THE methods previously adopted for the insect transmission of the viruses causing swollen-shoot disease of cacao (*Theobroma cacao* L.) employed seedlings usually between six and twelve months old. An improved technique utilizes the seed as a test plant and a watch-glass or specimen tube as a cage, a method which might be applicable to experiments with viruses of other plants with exalbuminous seeds.

The cacao bean is taken from the ripe pod, the testa removed, and one cotyledon dissected away to expose the convoluted surface of the other. Insects which have fed on an infected plant for the required time are transferred with a brush to the bean, placed in a solid or 'block' watch-glass. Humidity can be maintained by a square of damp filter paper under the cover of the watch-glass, and after the desired feeding time, the insects are killed with nicotine solution. The beans are then planted in sterilized sand in an insect-proof house. Germination is not affected, and symptoms usually appear in the first or second pair of foliage leaves, occasionally in the third. The vectors used successfully in these experiments are the mealy-bugs, *Pseudococcus citri* Risso, *Ps. njalensis* Laing, and *Ferrisia virgata* Ckll., which feed readily on the beans not only of cacao but also of *Cola* spp., and of *Phaseolus vulgaris*.

The use of beans in small containers has numerous advantages over that of larger plants in pots. The movement of the vectors is controlled, and feeding periods can be observed conveniently with a microscope. Complete exclusion of other insects is ensured without the necessity for cages, and a large number of test plants can be accommodated in a small space in the laboratory. Unless the virus under investigation is seed-transmitted, when care would be taken to ensure an uninfected source of seed, any chance of prior infection of the test plants is eliminated, together with the difficulty of maintaining a constant source of virus-free plants of the correct size and condition for vector experiments. Not the least important advantage of the method as applied to cacao is a shortening of the mean period between the time of infection and of the development of symptoms, and a reduction in its variation between plants. In experiments using large plants, the interval between inoculation and the appearance of symptoms depends on the periodicity of growth-flushes, which differs greatly with season and also between plants, resulting in latent periods varying between three weeks and four months. When seed is used, symptoms are usually visible in the leaves about three weeks after the beans were infested.

The method can be modified for testing suspected vectors found in natural outbreaks of virus diseases far distant from the laboratory. Peeled cacao beans