Decomposition of *tert*-Butyloxycarbonylamino Acids during Activation[1]

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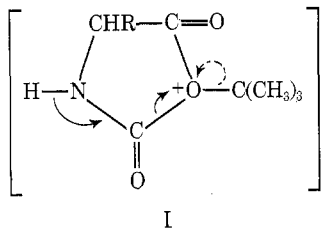
The formation of ninhydrin-positive impurities on reaction of *tert*-butyloxycarbonyl (Boc) amino acids with dicyclohexylcarbodiimide (DCC) was reported earlier.[2,3] The presence of ninhydrin-positive materials, presumably free amines, in solutions that contain a large excess of an acylating agent such as *O*-acylisourea derivatives[4] was intriguing. The free amines should be acylated under such conditions. An examination of the reaction mixture composed of equimolecular amounts of Boc-alanine and DCC in dichloromethane revealed that the ninhydrin-positive materials are not present as such in the mixture, but form on exposure to moist air or on contact with the chromatographic medium, thin layers of silica gel or paper. This observation not only resolves the apparent conflict of the simultaneous presence of amines and acylating agents, but also supports our earlier assumption that *N*-carboxyanhydrides (NCA's) are intermediates in the decomposition.

While the amount of the by-products allows their detection by the sensitive ninhydrin reaction, it turned out to be insufficient for a demonstration of the assumed intermediate NCA through ir spectra. Attempts to reveal the presence of an NCA through reaction with valyl polymer[5] or valine *tert*-butyl ester also failed.

Ninhydrin-positive spots were revealed on paper chromatograms[6] when reaction mixtures containing DCC and Boc derivatives of any of the 20 amino acids that occur in proteins was applied. The same pattern of spots could be observed: the most intense spot was that of the amino acid itself, a weak spot was found to correspond to the dipeptide consisting of two residues of the same amino acid, a spot at the origin suggested a polymer (or an aminoacyl derivative of cellulose), while a fast-moving species was identified as the *tert*-butyl ester of the amino acid. The outcome of the reaction was independent of the solvent. The same pattern, albeit in different intensities, was observed when toluene, dichloromethane, chloroform, acetonitrile, ethyl acetate, tetrahydrofuran, dimethylformamide, dimethyl sulfoxide, or hexamethylphosphoramide was used. The method of activation also could be varied. When, instead of DCC, ethyl chloroformate,[7] Woodward's reagent,[8] or EEDQ,[9] all in the presence of equimolar amounts of triethylamine, were applied, the decomposition products remained the same. None of these methods of activation affected benzyloxycarbonylamino acids. On the other hand, when a solution of Boc-alanine in ethyl acetate was treated with phosphorus pentoxide, evaporation of the decanted solution left the crystalline NCA of alanine, that after sublimation was identified by its characteristic ir spectrum and by its readiness to form, on exposure to water, polyalanine.

The formation of amino acid *tert*-butyl esters can be rationalized by the attack of the oxygen of the *tert*-butyloxy group on the carbonyl of the activated carboxyl group (I). The resulting oxonium intermediate could produce both an NCA that yields the free amino acid, the dipeptide, and also an isocyanate, the *N*-carbonylamino acid *tert*-butyl ester that in turn is hydrolyzed—on the chromatographic paper—to the amino acid *tert*-butyl ester.



I

Experimental Section

Reaction of Boc-Ala with DCC. To a solution of Boc-Ala (95 mg) in CH_2Cl_2 (2 ml), DCC (103 mg) was added at room temperature. From time to time, spots were applied to filter paper (Whatman No. 1) and stained with a 0.3% solution of ninhydrin in acetone. A purple spot developed after about 10 min. The same reaction was carried out also on a porcelain spot plate in a desiccator over P_2O_5. Ninhydrin (3 drops of above-mentioned solution) was added, and the mixture stored at room temperature. No purple color was observed until about 24 hr later, when the mixture was exposed to moist air. Then a positive reaction could soon be observed.

The formation of ninhydrin-positive by-products on filter paper was also found in a series of tests in which solvents other than dichloromethane (cf. introduction) were used.

Reactions of Boc-Ala with Activating Reagents. Boc-L-Ala (19 mg) in tetrahydrofuran (1 ml) was treated with triethylamine (TEA, 0.015 ml) and ethyl chloroformate (0.010 ml). A spot on filter paper gave a positive reaction with ninhydrin. A control mixture with benzyloxycarbonyl-L-alanine (Z-L-Ala) instead of Boc-L-Ala gave no reaction with ninhydrin.

Boc-L-Ala (19 mg) in CH_2Cl_2 (1 ml) was allowed to react with TEA (0.015 ml) and EEDQ[9] (25 mg). A positive ninhydrin reaction was observed on filter paper, but none in the control experiment with Z-Ala.

Similarly, Boc-L-Ala (19 mg) in CH_3CN (1 ml), when treated in the presence of TEA (0.015 ml) with Woodward's reagent K[8] (25 mg), gave a purple spot with ninhydrin on filter paper. The parallel experiment with Z-Ala produced no ninhydrin-positive material.

In all three experiments with Boc-L-Ala, a similar pattern was observed on descending paper chromatograms in the solvent system 1-butanol–acetic acid–water (4:1:5, upper phase).[6] The principal ninhydrin-positive spot corresponds to alanine; a somewhat faster moving component was identified as alanylalanine. A fast-moving spot was also observed (cf. below). In the experiment with ethyl chloroformate as activating reagent, an additional ninhydrin-positive spot was found at the origin.

Identification of the By-products with the Amino Acid Analyzer. A mixture of Boc-L-Ala (76 mg) in CH_2Cl_2 (3 ml) was neutralized with TEA (0.060 ml) and allowed to react with DCC (90 mg). After standing overnight at room temperature, the crystals were removed by filtration and H_2O (1 ml) was added to the filtrate. The CH_2Cl_2 layer was evaporated, and the residual aqueous solution was diluted to 3 ml with a pH 2.2 buffer. A portion (1 ml) of this solution was applied to the long column of a Beckman-Spinco 120C amino acid analyzer.[10] The largest peak corresponded to alanine; a significant peak eluted at 149 min was identified as alanylalanine by comparison, via elution times, with an authentic sample. For confirmation, a second sample (1 ml) was applied, together with authentic L-Ala-L-Ala. A third sample (1 ml) was applied to the short column of the amino acid analyzer. A peak emerged at 62 min; an authentic sample of L-alanine *tert*-butyl ester appeared exactly at that elution time. The presence of alanine *tert*-butyl ester was confirmed also on TLC (cellulose powder, 1-butanol–acetic acid–water, 4:1:1).

***N*-Carboxyanhydride from Boc-Ala.** To a solution of Boc-Ala (0.40 g) in EtOAc (20 ml), P_2O_5 (2.2 g) was added in small portions. The solution was spotted on filter paper and stained with ninhydrin: a strong positive reaction was observed. After 1 hr at room temperature, the mixture was filtered on a dry sinter-glass filter and the filtrate was evaporated to a crystalline residue (0.20 g) which in the ir lacked the urethane carbonyl band of the starting material, and showed two new carbonyl bands at 1780 and 1864 cm^{-1}. A sample was crystallized from ethyl acetate–petroleum ether (bp 50–70°), mp 92° (lit.[11] mp 92°). The NMR spectrum ($CDCl_3$) confirmed the absence of the *tert*-butyl group: only the signals of the CH_3, α-CH, and NH protons were present. The product sublimed as a single compound at 50° (0.1 mm). When treated with H_2O, evolution of gas could be observed, followed by the separation of insoluble polyalanine.

Registry No.—Boc-L-Ala, 15761-38-3; DCC, 538-75-0; EEDQ, 16357-59-8; Woodward's reagent K, 4156-16-5; ethyl chloroformate, 541-41-3.

References and Notes

(1) This study was supported by grants from the U. S. Public Health Service (NIH AI-07515 and AM-12473).
(2) The preliminary study was made in collaboration with Dr. J. T. Sheehan of The Squibb Institute for Medical Research; cf. M. Bodanszky in "Peptides, 1971", H. Nesvedba, Ed., North-Holland Publishing Co., Amsterdam, 1973, p 81.
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Use of (2,3-Dihydro-2-oxo-1*H*-1,4-benzodiazepin-3-yl)-phosphonic Acid Esters as Novel "Wittig Reagents"

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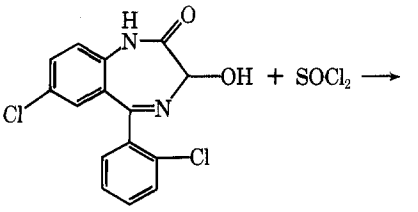
Because of our interest in the 1,4-benzodiazepine field, we sought a convenient method for the preparation of various useful lorazepam (**1**)[1] derivatives having functional substituents at the 3 position. One of our first thoughts was to prepare the 3-ketone and 3-methylene derivatives of **1** and use these groups as reactive intermediates. Only one paper[2] has described the preparation of any 1*H*-1,4-benzodiazepine-2,3-diones, and these preparations required the use of ruthenium tetroxide, which on any large preparative scale would be prohibitively expensive (5 g/$195.00). The preparation of 3-methylene-2*H*-1,4-benzodiazepin-2(3*H*)-ones has not been described. Instead of using this oxidation approach for the 3-keto type compounds, we decided to try making a "Wittig–Horner" type reagent from the benzodiazepine itself and using this reagent for the preparation of our desired intermediates. We found that **1** was easily converted to its corresponding 3-chloro derivative (**2**) with $SOCl_2$.[3] Condensation of **2** with $P(OMe)_3$ and $P(OEt)_3$ gave respectively **3** and **4**, by an Arbuzov–Perkow reaction.[4-6] Both **3** and **4** were methylated on the amide nitrogen by sodium hydroxide and dimethyl sulfate, giving respectively **5** and **6**. The acidic 3 carbon adjacent to the phosphorus was not methylated, at least on **3**, as evidenced by the $P-H_3$ coupling of **3** which is still present in the product **5**. Presumably this was also true in methylation of **4** to **6**, because **6** behaved like a Wittig reagent and the exchangeable NH of **4** disappeared. During one attempt to methylate the nitrogen of **3** with sodium hydride and methyl iodide in DMF, only **7** was isolated. Apparently the sodium iodide formed from the methylation on nitrogen caused an anionic demethylation of one of the phosphate OMe groups.[7]

The phosphonate carbanion of **6** was prepared in 1,2-dimethoxyethane with sodium hydride,[8] and reaction with gaseous formaldehyde readily gave **8**. Surprisingly, in spite of the apparent stability of phosphonate carbanions to oxygen,[4] reaction of the sodium salt of **6** with oxygen readily gave **9**.



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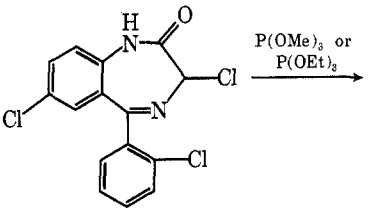

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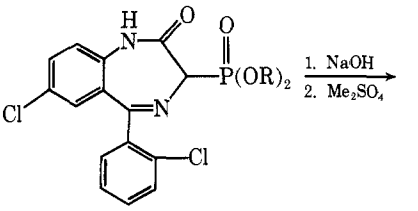

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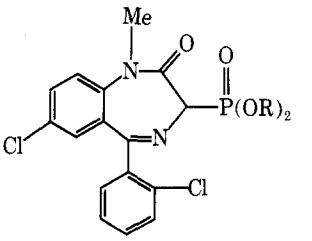

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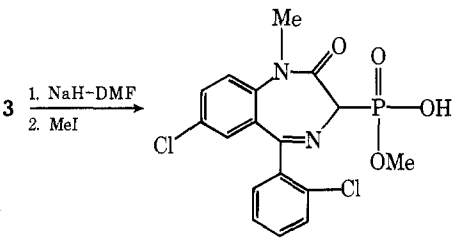
6, R = Et



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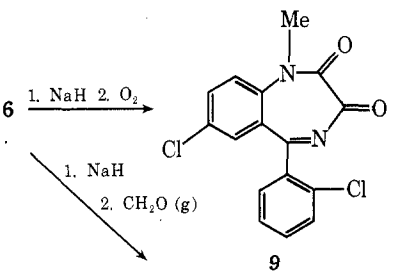

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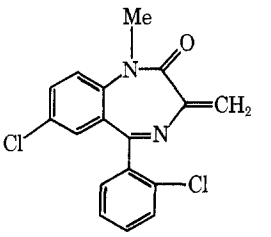

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