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(52) The distillation leaves a waxy residue from which a crystalline material (mp 65–67°) was isolated by recrystallization from ether containing a trace of chloroform. The ir spectrum of this material is similar to that of **18** but shows a greatly attenuated OH band and a small shift of the carbonyl absorption to 1725 cm^{-1}.
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(54) The material can also be purified by sublimation in vacuo at <50°.

Side Reactions in Peptide Synthesis. II.[1] Formation of Succinimide Derivatives from Aspartyl Residues

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Received April 7, 1975

Acylation of peptides in which the side chain of an aspartyl residue is unprotected can result in the formation of succinimide derivatives. The presence of base enhances the formation of such by-products: the carboxylate attacks the carbonyl of active esters and forms a reactive intermediate, probably a mixed anhydride, that in turn acylates the adjacent amide. This is a particularly serious side reaction in acylation with activated derivatives of glycine. The observation that substituted phenols liberated in active ester reactions can combine with tertiary bases and thus liberate the free side-chain carboxyl groups from their salts is also reported.

In our continued effort toward the total synthesis of the gastrointestinal hormone cholecystokinin,[2] synthesis of the N-terminal octapeptide was reported earlier.[3] Preparation of peptides corresponding to C-terminal sequences of this hormone was also undertaken and involved several intermediates that were used in synthesis of gastrin[4,5] and caerulein,[6] peptides which have C-terminal sequences closely related to that of cholecystokinin, and also in the synthesis of a biologically active dodecapeptide portion of cholecystokinin.[7] The initial steps of our synthesis are summarized in Scheme I. The aspartyl residue in the penultimate position was incorporated as the β-benzyl ester, but the side-chain protection was removed from the resulting dipeptide amide. Thus, from there on the synthesis was continued with amino components in which the carboxyl group of the aspartyl residue remained unprotected. This approach, applied also in other laboratories,[4,7] seemed to offer certain advantages. Protection of a side-chain carboxyl in the form of *tert*-butyl ester requires prolonged acidolysis at the deprotection stage; this was undesirable because of the presence of several acid-sensitive residues (tryptophan, tyrosine *O*-sulfate) in cholecystokinin. Benzyl ester protection was also considered but the presence of methionines in the sequence made it questionable whether or not hydrogenolysis can be applied for deblocking.[8] Last, but not least, a free carboxyl was thought to be less likely to lead to aminosuccinimide derivatives[9] (Chart I) than β esters of aspartic acid. Early removal of the benzyl ester protection, prior to the incorporation of the first methionine residue, was expected to circumvent these difficulties.

Scheme I
Synthesis of the Protected Pentapeptide VI

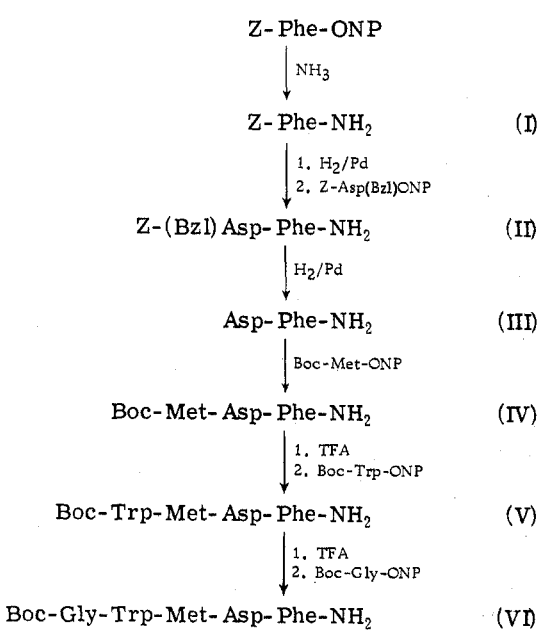
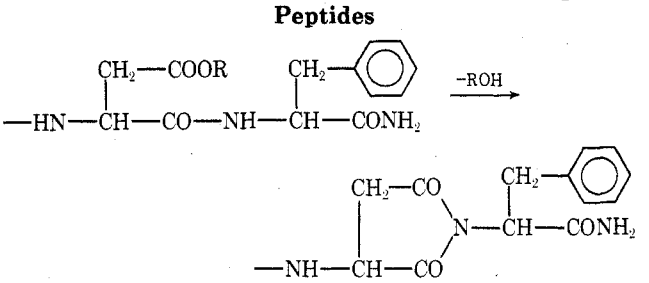
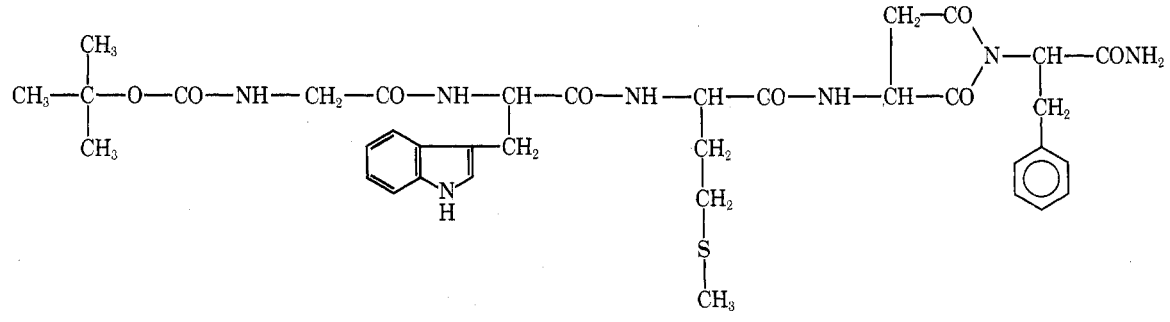


Chart I
Formation of Succinimide Derivatives from Aspartyl Peptides



In the synthesis of the protected tetrapeptide amide V and the protected pentapeptide amide VI, the formation of several by-products was observed. Most of these by-products represented only minor impurities. The presence of slow moving, polar materials on thin layer plates was attributed to air oxidation, producing peptides in which the methionine residues were oxidized to sulfoxides or sulfones. These impurities could be readily removed by recrystallization of the crude products. On the other hand, some less readily explained by-products could also be detected in the protected tetra- and pentapeptide derivatives. These products seemed to be less polar, since they moved faster on the chromatograms than the desired intermediates. In the step leading from the tetrapeptide derivatives to the protected

Chart II
Structure of the Succinimide Derivative VII Formed from the Protected Pentapeptide VI



VII

pentapeptide amide VI, a faster moving impurity was present in significant quantity, in some experiments exceeding the amount of the desired product. Therefore, an examination of this by-product (VII) became necessary.

Isolation of the major by-product VII by countercurrent distribution and further purification by chromatography on a silica gel column provided a homogeneous material that had the same amino acid composition as the (protected) pentapeptide amide VI. Their uv and NMR spectra also were quite similar; thus only some subtle difference could exist between the two compounds. In the ir spectrum of VII, a weak but definite carbonyl band at 1790 cm^{-1} suggested that a succinimide derivative[10] was at hand. Indeed, the ring could be opened by the action of ammonia or of alkali in alcohol. The values of elemental analysis and the electrophoretic mobility of the deblocked peptide substantiated the assignment of structure VII (Chart II) to this material.

The unexpected ring closure prompted a series of experiments aimed at the clarification of the reasons for this side reaction. These experiments (Table I) demonstrated that compound VII was present as a minor product if 1 mol of base was added to the tetrapeptide amide trifluoroacetate, an amount enough to liberate the amine from its salt, but not to form a salt with the carboxyl. With 2 mol of base, the formation of VII is enhanced. The effect of *tert*-butyloxycarbonylglycine active esters was similar. An excess of active ester was found to be harmful independently of whether *p*-nitrophenyl,[11] *o*-nitrophenyl,[12] or 2,4,5-trichlorophenyl[13] esters were used. We had to assume that the carboxylate as a nucleophile attacks the electrophilic carbonyl of the active ester and the resulting mixed anhydride intermediate acylates the amide group. A competition between amino groups and carboxylates for the carbonyl of active esters resulting in undesired acylation (e.g., acetylation) products was noted earlier.[14] The presence of base enhances this side reaction by increasing the concentration of carboxylate. Excess active ester has similar influence on the outcome of the two competing reactions. Also, when compound VI was treated with equimolar amounts of diisopropylethylamine and *tert*-butyloxycarbonylglycine *p*-nitrophenyl ester, the formation of significant amounts of the succinimide derivative VII was clearly visible on thin layer chromatograms. The observation that succinimide derivatives formed only to a minor extent in the incorporation of tryptophan, but that the side reaction became a major contributor when active esters of *tert*-butyloxycarbonylglycine were used for acylation, suggests that the side chain of the amino acids hinders the attack of carboxylate on the active ester carbonyl. In glycine, the absence of a side chain allows a more ready approach of the nucleophile, the carboxylate group. In the continuation of the chain lengthening, incorporation of methionine, tyrosine, and aspartic acid β-*tert*-butyl ester was accompanied with the formation of nonpolar by-products, but only to a small extent. In subsequent preparations of the protected pentapeptide VI, only 1 mol of tertiary base was applied and only a slight excess of active ester, the latter being added slowly in several portions. The yield and the quality of the product were superior to those experienced earlier.

Table I
The Effect of the Amount of Tertiary Base and of Excess Active Ester on the Formation of Succinimide Derivative VII

Tube no.	DIEA (0.4 *M*), ml	Boc-Gly-ONP % excess	Yield of crude product, g	Mp, °C	Ratio of VI:VII
1	0.5	20	0.14	163–167	5:1
2	1.0	20	0.13	140–150	5:2
3	1.0	20	0.12	138–145	5:4
4	1.0	100	0.11	128–135	1:5
5	0.5	100	0.14	130–145	5:3

In the synthesis shown in Scheme I, an additional observation was made. After deblocking with trifluoroacetic acid, the trifluoroacetates of the tripeptide and tetrapeptide amides were treated with 2 mol of tertiary base (diisopropylethylamine[15]). One mole was added for the liberation of the free amine from the trifluoroacetate salt, the second for the purpose of converting the free carboxyl group of the aspartyl residue to a carboxylate. After completion of the acylation, the products were isolated by removal of the solvent in vacuo and trituration of the residue with ether, then with ethyl acetate and ether. No acids were applied in the process and yet the products were not the expected diisopropylethylammonium salts of protected peptides, but essentially the free acids. This was shown by melting points, comparisons with authentic samples, and particularly by NMR spectra. We had to assume that the phenols (*p*-nitrophenol, 2,4,5-trichlorophenol) liberated from the active esters are responsible for this "acidification". The acidities of *p*-nitrophenol and of 2,4,5-trichlorophenol are too weak to account for such an effect. A satisfactory explanation was suggested by an earlier observation[16,17] of the formation of a crystalline complex from *p*-nitrophenol and pyridine. Indeed, an examination of washings from the crude products revealed complexes of *p*-nitrophenol with diisopropylethylamine and of 2,4,5-trichlorophenol and diisopropylethylamine. With triethylamine, similar complexes were formed. Treatment with *p*-nitrophenol removed diisopropylethylamine from the salt of compound VI, prepared from the components. The complexes could be identified on thin layer plates by their uv absorption and, in the case of *p*-nitrophenol, by their bright yellow color. From a practical point of view, these

observations raise the question whether or not it was necessary to add tertiary base to the reaction mixture with the intention to convert the carboxyl group to a carboxylate. During the acylation process, the gradually appearing substituted phenol at least partially liberated the free carboxyl group from the carboxylate, and still the reactions proceeded to completion. Thus, the free carboxyl of the aspartyl side chain did not prevent—by protonation—the acylation of the amine. Indeed, in a subsequent experiment, the protected tripeptide amide IV (Scheme I) was successfully prepared from the L-aspartyl-L-phenylalanine amide (III) by acylation with protected methionine active ester, without the addition of any tertiary base. Similarly, when in the preparation of the pentapeptide amide VI only 1 mol of base was used, enough to liberate the amine from its trifluoroacetate salt, but no base was added because of the presence of a free carboxyl in the amino component, the results were quite satisfactory. Thus the addition of tertiary amine for the neutralization of the free side chain carboxyl group of an aspartyl residue is not only unnecessary, but in fact disadvantageous.

The results of these studies necessitate a reconsideration of the use of unprotected side-chain carboxyls in peptide synthesis. They also show that by avoiding an excess on active esters and by using tertiary base only for the liberation of the amine from its salts, the formation of succinimide derivatives can be kept at a minimum.

Experimental Section[18]

Capillary melting points are reported uncorrected. On thin layer chromatograms, the protected peptides were revealed by *tert*-butyl hypochlorite–KI reagents[19] and by charring.[20] Tryptophan-containing peptides were detected by Ehrlich reagent. Peroxide-free ether was used; reagent-grade DMF was dried over a molecular sieve (Linde Type 4A). For amino acid analysis, samples were hydrolyzed with constant boiling HCl in evacuated, sealed ampoules at 110° for 16 hr and analyzed by the Spackman–Stein–Moore method.[21]

Benzyloxycarbonyl-L-phenylalanine Amide (I). Benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester[22] (8.4 g, 20 mmol) was dissolved in tetrahydrofuran (50 ml). Ammonia was passed over the stirred solution for 2 hr. The solvent was removed in vacuo, and the residue was triturated with ether, filtered, and dried in vacuo. The amide weighed 5.5 g (92%), mp 163–164° (lit.[5] mp 161–162°, lit.[4] mp 164–165), $[\alpha]^{25}D$ −6.3° (*c* 2, DMF).

Benzyloxycarbonyl-(β-benzyl)-L-aspartyl-L-phenylalanine Amide (II). A sample of I (9.0 g, 30 mmol) was dissolved in 95% ethanol (450 ml) by gentle warming. After cooling, 1 *N* HCl (30 ml) was added, and the mixture was hydrogenated in the presence of 10% Pd on charcoal catalyst (1.8 g). The catalyst was removed by filtration and the solvents by evaporation in vacuo. The residue was dissolved in DMF (69 ml) and DIEA (4.8 ml, 30 mmol) was added, followed by benzyloxycarbonyl-β-benzyl-L-aspartic acid *p*-nitrophenyl ester[23] (15.8 g, 33 mmol). Next day the ninhydrin reaction was negative. The solvent was removed in vacuo, and the residue was triturated with ethyl acetate (100 ml) and filtered. The solid was suspended in water (200 ml), stirred for 15 min, filtered, and dried: yield 14.7 g (97%); mp 170–171° (lit.[5] mp 170–171°); $[\alpha]^{25}D$ −22.6° (*c* 2, DMF) [lit.[5] −25.9° (*c* 1, DMF)].

In a second experiment, the corresponding *o*-nitrophenyl ester[24] was used with similar results.

***tert*-Butyloxycarbonyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (IV). A.** Compound II (14.5 g, 29 mmol) was dissolved in 95% aqueous acetic acid (240 ml) and hydrogenated in the presence of 10% Pd on charcoal catalyst (3 g) for 6 hr. The solution was filtered through a filter precoated with Celite (3.0 g) and charcoal (1.0 g), evaporated in vacuo, and reevaporated with a small quantity of water (5 ml). This was repeated three more times. The resulting aspartylphenylalanine amide (III) was used without purification. It was suspended in DMF (200 ml), and DIEA (4.8 ml) was added. *tert*-Butyloxycarbonyl-L-methionyl *p*-nitrophenyl ester[25] (12.0 g, 32.4 mmol) was added to the suspension; soon a clear solution formed. After about 16 hr, the ninhydrin reaction became negative. The solvent was evaporated in vacuo, and the residue was thoroughly triturated with ethyl acetate (20 ml) and ether (250 ml), stirred for 20 min with this solvent mixture, filtered, and dried to give 10.75 g (70%), mp 206–208° dec. A sample was recrystallized from EtOH, mp 211–212° dec (lit.[5] mp 209–210° dec), $[\alpha]^{25}D$ −41.9 (*c* 2.0, DMF) [lit.[5] −39.3° (*c* 1, DMF)].

B. Compound III (prepared from 375 mg of compound II as described above) was suspended in DMF (6 ml), and Boc-Met-ONP (0.33 g) was added to the stirred suspension. Gradually a clear solution formed. After about 48 hr, the solvent was removed in vacuo and the residue was triturated with ether. The protected tripeptide amide IV (0.32 g, 85%) melted at 206–208° and was homogeneous on TLC.

***N-tert*-Butyloxycarbonyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (V).** A sample of the protected tripeptide amide IV (8.3 g, 16.3 mmol) was dissolved in distilled TFA containing 5% anisole (30 ml). After 30 min in an ice bath, under N_2, the TFA was evaporated in vacuo, and the residue was triturated with ether, filtered, washed with ether, and dried to yield the tripeptide amide trifluoroacetate (8.3 g). This was dissolved in DMF (90 ml), and DIEA (5 ml) was added, followed by *tert*-butyloxycarbonyl-L-tryptophan *p*-nitrophenyl ester[26] (8.0 g, 18.8 mmol). 1-Hydroxybenzotriazole[27] (2.3 g) was added to the suspension; a further 10 ml of DMF was used for the rinsing. The stirred reaction mixture became clear in about 5 min. After 2 hr, the ninhydrin reaction was negative. (Without the catalyst, the reaction required 72 hr.) The solvents were removed in vacuo, and the residue was triturated with ethyl acetate (20 ml) and ether (250 ml), filtered, washed with ether, and dried, 10.2 g (90.2%), mp 194–195° dec. A sample was crystallized from ethanol, mp 212–213° (lit.[5] mp 209–210° dec, $[\alpha]^{25}D$ −35.0° (*c* 2.0, DMF) [lit.[5] −35.7° (*c* 1, DMF)]. Amino acid analysis: Asp, 1.05; Met, 1.0; Phe, 0.98.

Anal. Calcd for $C_{34}H_{44}N_6O_8S$: C, 58.6; H, 6.4; N, 12.1. Found: C, 58.4; H, 6.2; N, 11.8.

With *tert*-butyloxycarbonyl-L-tryphophan 2,4,5-trichlorophenyl ester, similar results were obtained.

***N-tert*-Butyloxycarbonylglycyl-L-tryptophyl-L-methionyl-L-aspartyl-L-phenylalanine Amide (VI). A.** The *tert*-butyloxycarbonyl group was removed from compound V (0.70 g, 1 mmol) with TFA (2 ml, containing 5% anisole) as described in the previous experiment. The trifluoroacetate salt was dissolved in DMF (8 ml) and DIEA (0.32 ml, 2 mmol) was added, followed by *tert*-butyloxycarbonylglycine *p*-nitrophenyl ester[28] (0.33 g, 1.1 mmol). The solution was kept overnight at room temperature, when the ninhydrin and fluorescamine[29] reactions became negative. The solvents were evaporated in vacuo, the residue was thoroughly triturated with ether (30 ml), and the supernatant was decanted. The residue was triturated with ethyl acetate (5 ml) and then ether (30 ml) was added. The suspension was stirred for 10 min and filtered. The solid was resuspended in ethyl acetate (10 ml), stirred for 10 min, and filtered, yield 0.60 g. Crystallization from ethanol yielded compound VI, which on TLC still showed a few minor spots, mp 187–190° dec. The yield of this reaction was found to be variable, sometimes as low as 40%. The purification of the product was difficult: it formed gels in several solvents.

B. To a sample of the tetrapeptide amide trifluoroacetate (0.71 g, 1 mmol) in DMF (8 ml), DIEA (0.16 ml) was added. The active ester, Boc-Gly-ONP (0.36 g, 1.2 mmol), was added in five portions at 90-min intervals, the first four portions 0.25 mmol each, the final addition 0.20 mmol. Before each addition, a sample (0.1 ml) was removed and diluted with ether, and the precipitate was separated by centrifugation and examined on TLC. After the last addition of active ester, the mixture was allowed to stand overnight and worked up as described in the previous experiments. The product (0.72 g) had mp 178–180° dec. After trituration with ethyl acetate (6 ml), the product (0.67 g, 93% corrected for sample) melted at 185–186° dec. The formation of only slight amounts of VII could be detected both during the reaction and in the crude product.

Preparation of the Diisopropylethylammonium Salt of VI. A crude sample of VI (1.7 g) was dissolved in a 1:1 mixture of chloroform and ethanol (50 ml), and DIEA (0.48 ml) was added. The solution was distinctly alkaline. The solvents were removed by a stream of N_2 and the residue was dried in a dessiccator. It was distributed through 46 transfers in a Craig apparatus with 10 ml upper and 10 ml lower phases of the system chloroform–methanol–water (3:2:1). On examination on TLC, tubes 18–26 showed the presence of the diisopropylethylammonium salt of VI as a single entity. These fractions were pooled, the solvents were removed, and the residue was dried to yield 0.94 g. The NMR spectrum of this compound revealed this to be the DIEA salt of VI, mp 125–

134° dec. Amino acid analysis: Asp, 1.0; Gly, 1.14; Met, 0.97; Phe, 1.0.

Anal. Calcd for $C_{44}H_{66}N_8O_9S$: C, 59.9; H, 7.5; N, 12.7. Calcd for $C_{44}H_{66}N_8O_9S \cdot 2H_2O$: C, 57.5; H, 7.7; N, 12.2. Found: C, 57.6; H, 7.3; N, 12.2.

Conversion of the Diisopropylethylammonium Salt of VI to the Free Acid VI by *p*-Nitrophenol. A sample of the DIEA salt of VI (7 mg) was suspended in ether (2 ml), followed by the addition of *p*-nitrophenol (3 mg). The ether solution was decanted, concentrated, and applied to a thin layer plate of silica gel. Ether was used for elution. The *p*-nitrophenol–DIEA complex was identified by comparison with an authentic sample. The ether-insoluble material melted at 195–197° dec and was similarly identified as VI.

Isolation of the Succinimide Derivative VII. The ethyl acetate soluble portions obtained from the crude reaction products of VI from several experiments (3.45 g) were distributed in a 60-tube Craig apparatus with 10-ml phases in the solvent system chloroform–methanol–water (3:2:1). After 80 transfers, on the basis of TLC chromatograms, fractions 0–3 (1.9 g), 4–6 (0.9 g), 7–10 (0.14 g), and 11–22 (0.35 g) were pooled. Fractions 4–6 contained the succinimide with some impurities. This material was chromatographed on a column of silica gel (20 g). First, chloroform was used for elution; 6-ml fractions were collected. After 25 fractions, the eluent was changed to a mixture of 2% methanol in chloroform. Purified VII was detected in fractions 75–149. These fractions were combined and solvents removed to leave a residue (550 mg) of compound VII in homogeneous (TLC) form, mp 131–136° dec, $[\alpha]^{25}D$ −76.2° (*c* 2, DMF containing 1% AcOH). On TLC in $CHCl_3$–MeOH (9:1) R_f 0.38, in EtOAC–pyridine–HOAC–H_2O (60:20:6:11) R_f 0.8. Amino acid analysis: Asp, 1.01; Gly, 0.96; Met, 1.01; Phe, 1.0.

Anal. Calcd for $C_{36}H_{45}N_7O_8S$: C, 58.8; H, 6.2; N, 13.3. Found C, 58.5; H, 6.1; N, 13.1.

Fractions 11–22 of the countercurrent distribution yielded *t*-Boc pentapeptide amide VI (0.35 g) in homogeneous form; mp 201–202° dec (lit.[4] mp 200–202° dec, lit.[6] mp 196°), $[\alpha]^{25}D$ −32.2° (*c* 2, DMF containing 1% AcOH) [lit.[4] −27.7 (*c* 2, DMF), lit.[6] −27 (*c* 1, DMF)]. Amino acid analysis: Asp, 1.15; Gly, 0.89; Met, 1.0; Phe, 1.0.

Anal. Calcd for $C_{36}H_{47}N_7O_9S$: C, 57.4; H, 6.3; N, 13.0. Found: C, 57.1; H, 6.4; N, 13.3.

Studies on the Formation of VII. A. The Effect of Different Active Esters. In three parallel experiments, the preparation of VI was carried out under identical conditions: 1 mmol of amino component, 2 mmol of DIEA, and 1.1 mmol of active ester in 8 ml of DMF with 2,4,5-trichlorophenyl ester, *o*-nitrophenyl ester, and *p*-nitrophenyl ester of *tert*-butyloxycarbonyl glycine as acylating agents. The crude products were stirred with EtOAC (5 ml), separated by centrifugation, dried, and weighed. The results are shown in Table II.

Table II
The Influence of Different Active Esters on the Formation of Compound VII

	With Boc-Gly-ONO	With Boc-Gly-OCP	With Boc-Gly-ONP
Wt of crude product, g	0.64	0.66	0.60
Mp of crude product, °C	100–125	100–125	140–150
Wt of purified material, g	0.35	0.38	0.39
Mp of purified material, °C	177–180	177–178	181–184

B. The Effect of the Amount of Base and of the Excess of Ester. Five aliquots (1 ml each) of a 0.2 *M* DMF solution of the trifluoroacetate, obtained by deprotection of the protected tetrapeptide amide VI in DMF, were placed in 40-ml centrifuge tubes equipped with a 24/40 standard tapered joint.[30] A 0.4 *M* solution of DIEA in DMF was added in amounts shown in Table I. To keep the concentration of the amino component identical in each experiment, DMF (0.5 ml) was added to tubes 1 and 5. The amount of active ester used (Boc-Gly-ONP) is shown in Table I. The reaction was allowed to proceed overnight. The solvent was removed in vacuo, each residue was triturated with ether (15 ml), the semisolid products were separated by centrifugation and decantation and triturated with ethyl acetate (3 ml), and the mixture was diluted with ether (15 ml). The crude products were separated again by centrifugation. Their weights and melting points are shown in Table I, and also their content on compounds VI and VII as estimated from the intensities of the respective spots on TLC.

C. Reaction of Boc-Gly-ONP and Boc-Met-ONP with VI in the Presence of DIEA. Compound VI (38 mg) was added to a 0.025 *M* solution (0.2 ml) of DIEA in DMF. This was divided into two equal portions. To one portion Boc-Gly-ONP (7.5 mg) was added, to the other Boc-Met-ONP (9.5 mg); DMF (0.1 ml) was used in each tube for rinsing. The solutions were kept overnight. The solvent was removed by a stream of nitrogen. The residues were triturated with ether (2 ml) and centrifuged and the ether solution was decanted. The remaining residues were examined on TLC. The formation of VII was observed in both cases, but was more pronounced in the experiment with Boc-Gly-ONP.

D. Opening of the Succinimide Ring. A sample (0.05 mmol) of compound VII was dissolved in 95% ethanol (2 ml). A 0.2 *N* NaOH solution (0.25 ml) was added, and the mixture was kept at room temperature for 90 min and then acidified with 0.2 *N* HCl (0.25 ml). Examination on TLC [silica gel, EtOAc–pyridine–AcOH–H_2O (60:20:6:11)] showed the disappearance of VII (R_f 0.80), the formation of VI (R_f 0.46), and an even larger amount of a new derivative with R_f 0.33, presumably the pentapeptide derivative containing a β-aspartyl residue.

A sample of VII (5 mg) was dissolved in MeOH (3 ml) and NH_3 was passed over the solution for 1 hr. After 2 days at room temperature, examination on TLC in the system described above revealed the disappearance of VII and the formation of two new compounds, with R_f values 0.68 and 0.63.

E. Electrophoresis. Samples of compounds VI and VII (40 mg each) were treated with TFA (0.5 ml) containing 5% anisole for 30 min at ice-bath temperature under a blanket of nitrogen. The TFA was removed in vacuo, the residues were triturated with ether, and trifluoroacetates were isolated by centrifugation. Samples were dissolved in 1 *N* AcOH and applied to Whatman No. 3 MM paper. Electrophoresis was carried out with the Savant flat plate apparatus, in a buffer of pyridine (300 ml), AcOH (11.5 ml), and H_2O (2700 ml) at 30 V/cm for 2.5 hr. After drying, the spots were revealed with ninhydrin; the pentapeptide amide corresponding to VI moved slightly toward the cathode, while the spot of deblocked VII moved further away from the origin, with about 40% of the mobility of lysine.

Acknowledgments. This study was supported by a grant from the U.S. Public Health Service (NIH AM-12473). Amino acid analyses were carried out by Mrs. Delores J. Gaut, elemental analyses by the Baron Consulting Co., Orange, Conn.

Registry No.—I, 4801-80-3; II, 5241-68-9; III, 5241-71-4; IV, 5920-14-9; V, 5235-21-2; V trifluoroacetate, 5908-10-1; VI, 5915-71-9; VI diisopropylethylammonium salt, 55701-83-2; VII, 55701-84-3; benzyloxycarbonyl-L-phenylalanine *p*-nitrophenyl ester, 2578-84-9; DIEA, 7087-68-5; benzyloxycarbonyl-β-benzyl-L-aspartic acid *p*-nitrophenyl ester, 55723-11-0; benzyloxycarbonyl-β-benzyl-L-aspartic acid *o*-nitrophenyl ester, 55701-85-4; *tert*-butyloxycarbonyl-L-methionyl *p*-nitrophenyl ester, 2488-18-8; Boc-Met-ONP, 41120-66-5; Boc-Trp *p*-nitrophenyl ester, 15160-31-3; Boc-Trp 2,4,5-trichlorophenyl ester, 15160-30-2; Boc-Gly *p*-nitrophenyl ester, 3655-05-8; Boc-Gly-ONP, 38606-09-6; *p*-nitrophenol, 100-02-7; Boc-Gly 2,4,5-trichlorophenyl ester, 7536-61-0.

References and Notes

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groups, it does not permit deblocking of *O*-benzyl serine residues.
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Carbon-13 Nuclear Magnetic Resonance Spectral Analysis of Quassinoid Bitter Principles[1]

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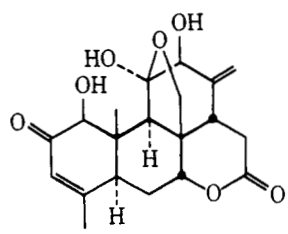
Received February 24, 1975

The ^{13}C NMR spectra of several types of quassinoid terpenic substances are presented and all chemical shifts assigned. The data are used for the corroboration of the structure of a new quassinoid principle.

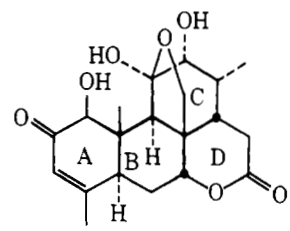
The bitter principles of the plant family Simaroubaceae are a group of structurally complex, highly oxygenated, triterpene degradation products which have attracted attention especially since the advent of ^1H NMR spectroscopy.[3] The latter and mass spectroscopy have been the major tools of structure analysis in this field of terpene chemistry in recent times. In view of the diagnostic power of ^{13}C NMR spectroscopy this new analytical method now has been utilized for the analysis of quassinoid compounds of several structure types and the data applied to the confirmation of the structure of a new substance from *Perriera orientalis* Courchet.

The ^{13}C NMR investigation was initiated by an inspection of the proton-decoupled as well as single-frequency, off-resonance decoupled spectra of ailanthone (**1**), chaparrinone (**2**), and glaucarubinone (**3a**), three compounds differing from each other only in rings C and D.

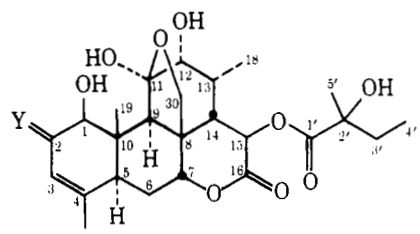
The chemical shifts of the carbonyl carbons of **1**, **2**, and **3a** are deduced from known ^{13}C NMR parameters of 2-cyclohexenones, δ-lactones, and branched esters.[1,4,5] Similarly, the olefinic carbon shifts are derived from those of 2-cyclohexenone and methylenecyclohexane models. The methyl shifts of the three compounds are based on the differentiation of the 4- and 10-methyl groups of **1** by the use of 1-methylcyclohexene as a model, and 13-methyl group of **2** being recognized by default and the two methyl groups of the side chain of **3a** differing from each other by their being the equivalent of neopentyl and homoneopentyl carbons. The 2.3-ppm difference of the chemical shift of C(18) in **2** vs. **3a** is a reflection of the δ effect[6] exerted by the interaction of the peri C(13) and C(15) substituents. As a comparison of the high-field C(19) shifts of compounds **1–3** with



1



2



3a, Y = O
b, Y = α-OH, β-H

those of substances possessing an 8β-methyl group instead of the oxymethylene bridge (vide infra) indicates, shielding of up to 2 ppm is due to the heterocycle spanning C(8) and C(11).

The methylene shifts of ailanthone (**1**) and chaparrinone (**2**) are distinguished easily from each other in view of one being associated with an expectedly low-field oxymethylene, another with a ketomethylene and, finally, one with an unsubstituted, upfield methylene function. In accord with conformations **4** and **5** for ailanthone and chaparrinone, respectively, one of the ketomethylene hydrogens, H(15β), experiences a peri interaction with C(18) in the lat-