evaporation of ether from the extract was dried by azeotropic distillation of water with benzene, then esterified with absolute methanol (50 ml) and concentrated sulfuric acid (1 ml). Most of the methanol was removed from the reaction mixture by codistillation with benzene and the mixture was neutralized with cold aqueous sodium carbonate solution. The aqueous layer was saturated with salt and extracted repeatedly with benzene. The combined benzene extracts were washed with water, dried, and concentrated by distillation of most of the solvent. In GLC examination[21] of product mixtures from aspartic acid, the esters of oxalic, fumaric, succinic, and maleic acids accounted for 97 ± 1% of the area under product elution peaks. A measured amount of dimethyl citraconate was added as an internal standard to ester mixtures for quantitative estimation of products.

Dimethyl esters of oxalic, fumaric, and succinic acids accounted for 70 ± 1% of the area under elution peaks in GLC of product mixtures from alanine–aspartic acid pyrolysis; several unidentified minor and trace products from alanine accounted for the remaining area. Dimethyl fumarate was used as an internal standard for quantitative estimation of dimethyl succinate. Results of GLC examination of the product mixture obtained from pyrolysis of alanine–fumaric acid were similar to those described for alanine–aspartic acid mixtures.

The identities of dimethyl esters of nonvolatile acid products were confirmed by isolation in preparative GLC[21] and comparison of their mass spectra with those of authentic samples and, except for dimethyl maleate, by means of infrared spectra and melting points.

In actual-scale control experiments, mixtures of succinic and fumaric acids were carried through the analytical procedure described above. The amounts of succinic and fumaric acids observed as dimethyl esters in GLC were 84 ± 2 and 82 ± 9% and the yield data for succinic and fumaric acids given in Tables I, III, and IV have been corrected accordingly. In a similar control experiment, not to scale, only moderate loss of maleic acid occurred during the analytical procedure; yields of this trace product were corrected using the somewhat arbitrary correction factor of 1.2.

Dimethylmaleic anhydride was removed from the hydrolyzed pyrolyzate by distillation with steam prior to continuous ether extraction of the nonvolatile acid products of pyrolysis. Gas–liquid chromatography of the organic material extracted from the steam distillate gave several trace peaks and one major elution peak, which corresponded in retention time to that of a purified sample of dimethylmaleic anhydride obtained from Aldrich Chemical Co. Dimethylmaleic anhydride was isolated by means of preparative GLC and its identity was confirmed by comparison of its mass spectrum, infrared spectrum, and melting point with those of authentic material.

Carbon-13 assays were done by the Mass Spectroscopy Center of the University of Kentucky.

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Registry No.—DL-Aspartic acid, 617-45-8; succinic acid, 110-15-6; dimethylmaleic anhydride, 766-39-2; methyl DL-2-acetamidopropionate, 26629-33-4; DL-alanine, 302-72-7.

References and Notes

(1) A. Pictet and M. Cramer, *Helv. Chim. Acta*, **2**, 188 (1919).
(2) K. R. Geisinger, T. C. Jones, and I. Schmeltz, *Tob. Sci.*, **14**, 89 (1970).
(3) L. D. Quin, W. George, and B. S. Menefee, *J. Assoc. Off. Agric. Chem.*, **44**, 367 (1961).
(4) I. Schmeltz and W. S. Scholtzhauer, *Tob. Sci.*, **6**, 90 (1962).
(5) Our analytical procedure was not suitable for quantitative estimation of oxalic acid, but this product seems not to have been formed in significant amounts in malic acid pyrolysis.
(6) W. T. Smith, Jr., S. P. Chen, and J. M. Patterson, *Tob. Sci.*, **19**, 53 (1975).
(7) K. R. Geisinger, T. C. Jones, and I. Schmeltz, *Tob. Sci.*, **14**, 89 (1970).
(8) A. Pictet, *Ber.*, **14**, 2648 (1881).
(9) L. D. Quin, W. George, and B. S. Menefee, *J. Assoc. Off. Agric. Chem.*, **44**, 367 (1961).
(10) I. Schmeltz and W. S. Schlotzhauer, *Tob. Sci.*, **6**, 90 (1961).
(11) See, for example, C. D. Hurd, *ACS Monogr.*, **No. 50**, 469 (1929), and references cited therein.
(12) J. U. Nef, *Justus Liebigs Ann. Chem.*, **298**, 202 (1897).
(13) R. Stolle, *Ber.*, **43**, 2471 (1910).
(14) H. Staudinger, *Ber.*, **44**, 543 (1911).
(15) C. A. Bischoff and P. Walden, *Justus Liebigs Ann. Chem.*, **279**, 118 (1894).
(16) C. D. Hurd, *ACS Monogr.*, **No. 50**, 430 (1929).
(17) C. D. Hurd and H. R. Raterink, *J. Am. Chem. Soc.*, **55**, 1541 (1933).
(18) A. Pictet, *Ber.*, **14**, 2648 (1881).
(19) Alanine had a negligible effect on the yield of succinic acid from aspartic acid, but, as noted earlier, a part of the aspartic acid may have been consumed in reducing alanine to propionic acid, which would have escaped detection in the analytical scheme employed, if formed.
(20) P. Karrer, K. Escher, and R. Widmer, *Helv. Chim. Acta*, **9**, 301 (1926).
(21) The column packing used was Silar 5CP on Gas-Chrom Q, supplied by Applied Science Laboratories Inc.; 3% liquid phase for analytical column packing, and 10% liquid phase for preparative GLC.

Study of Benzhydrylamine-Type Polymers. Synthesis and Use of *p*-Methoxybenzhydrylamine Resin in the Solid-Phase Preparation of Peptides[1]

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Starting with the phenylketopolystyrene–1% divinylbenzene intermediate, two routes have been compared for the synthesis of phenylaminomethylpolystyrene–1% divinylbenzene (benzhydrylamine-type polymer), useful for the preparation of C-terminal amide peptides by solid-phase synthesis. The desired primary amine containing polymer can best be obtained via the Leuckart reaction, while reduction of the oxime intermediate with metal hydrides leads to a large percentage of secondary amine. The Demjanov reaction has been adapted for the analysis of primary and secondary amine content of the polymers. Anisylaminomethylpolystryrene–1% divinylbenzene has also been synthesized by the Leuckart reaction, characterized with respect to primary and secondary amine content, and its usefulness illustrated by the synthesis of the hypothalamic hormone, Thyroliberin (TRH), and a series of model peptides.

In 1970 Pietta and Marshall[2] introduced into solid-phase peptide synthesis[3] a resin based upon a phenylaminomethylpolystyrene–2% divinylbenzene structure (benzhydrylamine resin), which attaches the growing peptide chain via a C-terminal amide bond to the polymeric support. The advantage over the utilization of a benzyl ester type linkage[4] for the

synthesis of peptides terminating in a carboxamide are the elimination of transesterification[5-8] and the possibility for the direct synthesis of peptides with a C-terminal carboxamide moiety containing aspartic and/or glutamic acid residues in the peptide chain.

A literature survey indicates that most syntheses using benzhydrylamine resin deal with the preparation of TRH, LH-RH, calcitonin, oxytocin, and their respective analogues, all of which terminate in either glycinamide or prolinamide.[9-16] Fortunately, peptides terminating in these two residues are removed from the benzhydrylamine resin in high yield by treatment with HF. However, experiences in our tides with a C-terminal phenylalanine residue are removed from benzhydrylamine resins at a low yield of 25–30%, although Pietta et al.[18] report a yield of 57% with the crude C-terminal tetrapeptide of gastrin, Trp-Met-Asp-Phe-NH_2.

The reason for the low yields obtained resides in the method recommended for the preparation of the benzhydrylamine resin. Pietta et al.[9] favor the synthesis of the benzhydrylamine resin via an oxime derivative which is reduced by metal hydride, although two additional routes starting with the phenylketo derivative of polystyrene–1% (or 2%) divinylbenzene copolymer have been explored (Figure 1). The route

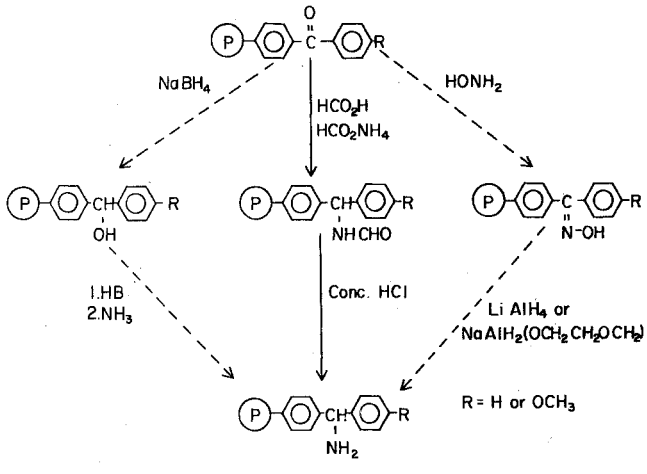


Figure 1. Synthetic routes to benzhydrylamine resin. The pathway indicated in solid lines was preferred in this study.

via the oxime may not have been the best choice, since it is a priori suspect to deleterious rearrangements. It has been reported[19,20] that the reduction of oximes can be accompanied by rearrangement involving a Curtius-type intermediate to give secondary amines, from which the final peptide cannot be removed by HF treatment.[21]

Another problem concerns the rate of acid-catalyzed cleavage of the peptide amide from the benzhydrylamine resin. The rate is directly proportional to the stability of the resultant carbonium ion on the polymer. Therefore, a *p*-anisyl-substituted resin—rather than a phenyl-substituted resin—would be expected to facilitate the release of the peptide amide.

In view of the above considerations the preparation and properties of benzhydrylamine resins, including the *p*-methoxy derivative,[2,18] were studied in some detail. The suitability of *p*-methoxybenzhydrylamine resin for the synthesis of peptides terminating in a carboxamide is investigated.

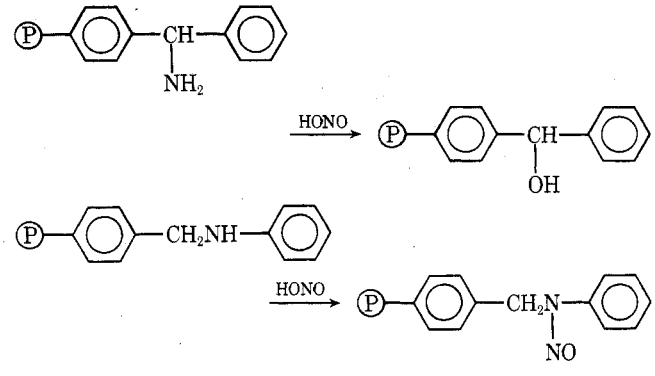
Results and Discussion

There are three synthetic routes to benzhydrylamine resin, starting from the common intermediate, phenylketo derivative, prepared by the Friedel–Crafts benzoylation of polystyrene–1% divinylbenzene copolymer (see Figure 1). For our work we have concentrated on the Leuckart and oxime reduction routes.

Three samples of benzhydrylamine resin were prepared from the same benzoylated polystyrene. One portion was converted to the oxime derivative and then reduced with Vitride according to the procedure described by Pietta et al.[9] Another sample of the oxime was subjected to Vitride reduction using a reversed sequence addition of reagents. A third portion of the benzoylated polystyrene was subjected to Leuckart reduction, followed by hydrolysis of the formylamine. The three products were analyzed for primary and secondary amine content by an adaptation of the Demjanov reaction.[22]

Primary amine will react with the nitrous acid to evolve N_2 and generate a secondary alcohol. Secondary amine will not diazotize but will nitrosate to yield a nitrosoamine derivative (Scheme I). Clearly, the nitrogen content of the polymer after

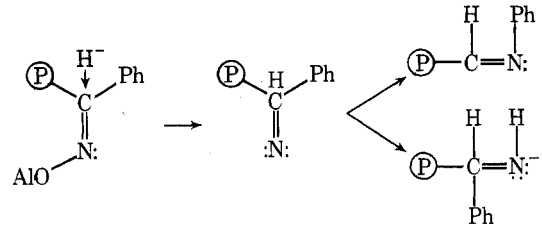
Scheme I



diazotization is a direct measure of the secondary amine content. Subtraction of the secondary amine value from the total amine yields the primary amine content of the resin.

The formation of secondary amine as a by-product of the hydride reduction of acetophenone oxime was first reported by Smith et al.[19] It was later suggested that hydroxylamines are intermediates in the hydride reduction of oximes.[20,23] We prefer the explanation of Graham and Williams[24] for the reduction of oximes with hydrides, which proceeds via the metal complex shown in Scheme II. In this case, the aluminate ion

Scheme II



is displaced either by attack of hydride ion (primary amine formation) or by phenyl migration (secondary amine formation). The results in Table I clearly indicate that the benzhydrylamine resin prepared via reduction of the oxime intermediate contains substantial amounts of secondary amine, while the resin with the highest primary amine content is obtained by the Leuckart reaction. For this reason, the *p*-methoxybenzhydrylamine polymer evaluated in this study for its usefulness in peptide synthesis was prepared via the Leuckart reduction of the *p*-anisoylated polystyrene–1% divinylbenzene copolymer. The resin obtained contained only 0.02 mequiv of secondary amine per gram of polymer and 0.6 mequiv/g of primary amine.

Table I. Comparison of Benzhydrylamine Resin Prepared by Leuckart Reaction or Oxime Reduction

Resin	Nitrogen, %	Total amine, mequiv/g	Nitrogen after diazotization, %	Secondary amine, mequiv/g	Primary amine, mequiv/g
Oxime I	1.33	0.95	1.40	0.50	0.45
Oxime II	1.36	0.97	1.70	0.61	0.36
Leuckart	1.22	0.87	0.06	0.02	0.85

Table II. Dipeptide Synthesis on *p*-Methoxybenzhydrylamine Resin

C-Terminal protected amino acid residue	Ninhydrin test after 1st coupling step	C-Terminal amino acid concn, μmol/mg	Ninhydrin test after second coupling step with Boc-Ala	Acid hydrolysis of dipeptide resin	
				C-terminal residue, μmol/mg	Ala concn, μmol/mg
Box-Gly	Negative	0.46	Negative	0.45	0.47
Boc-Pro	Negative	0.47	Negative	0.48	0.49
Boc-Phe	Negative	0.45	Negative	0.46	0.45
Boc-Val	Negative	0.53	Negative	0.51	0.49
Boc-Glu(OBzl)	Negative	0.47	Negative	0.51	0.46

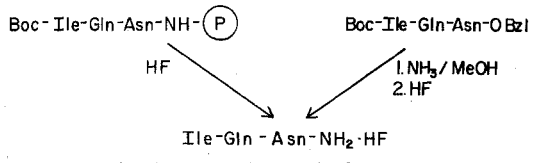


Figure 2. Methods of preparation of Ile-Gln-Asn-NH_2 by classical and solid-phase procedures.

A selected number of model dipeptides were synthesized on the *p*-methoxybenzhydrylamine resin in order to test for stability of the C-terminal amide bond during the removal of the *N-tert*-butyloxycarbonyl group with 33% v/v trifluoroacetic acid in methylene chloride. Samples of resin substituted with the first N-portected amino acid (X) were subjected to acid hydrolysis and amino acid analysis[25] as were the Boc-Ala-X-resin dipeptide products. From the results listed in Table II it is apparent that the C-terminal amide bond is stable to Boc-deprotection conditions. All of the five protected amino acids investigated substituted on the resin with ease and in high yields.

In view of the reported low recovery from benzhydrylamine resin[17,18] of peptides terminating in phenylalanine the Boc-Ala-Phe-*p*-methoxybenzhydrylamine polymer was subjected to treatment with anhydrous HF. The dipeptide Ala-Phe-NH_2·HF was recovered in 92% yield. The substitution level on the resin for Phe is 0.45 mequiv/g and for Ala 0.46 mequiv/g (Table II). After HF cleavage of the dipeptide, the residue resin was subjected to acid hydrolysis[25] and amino acid analysis. The amino acid levels found are Phe, 0.02 mequiv/g; Ala, 0.02 mequiv/g. This is indicative of at least a 95% cleavage of dipeptide from the resin.

To further demonstrate the usefulness of the *p*-methoxybenzhydrylamine resin, the synthesis of the hypothalamic hormone Thyroliberin (TRH)[26] was undertaken. The synthetic hormone was recovered in 78% yield after purification, and had a biological activity identical with that of the TRH standard when tested in the in vitro rat pituitary assay.[27]

The *p*-methoxybenzhydrylamine resin was further evaluated by introducing the first amino acid residue onto the resin via an activated ester condensation. The derivative synthesized was Boc-Ile-Gln-Asn-resin and the crude tripeptide was recovered in 94% yield, 65% after crystallization from water/2-propanol. Inasmuch as Ile-Gln-Asn-NH_2 is a possible candidate in our continued search for the MSH-releasing factor[28,29] it was deemed necessary to confirm the physical chemical properties of the tripeptide prepared by an independent method using classical peptide synthesis (Figure 2). The benzyl ester Boc-Ile-Gln-Asn-OBzl[30] was converted to the amide with ammonia in methanol and then deprotected with HF in the presence of anisole. Both classical and solid-phase products were identical.

Experimental Section

***p*-Anisoylation of Copolystyrene–1% Divinylbenzene.** To a stirred suspension of 50 g of polystyrene resin cross-linked with 1% divinylbenzene (Bio-Beads, X-1, 200–400 mesh, Bio-Rad Corp., Richmond, Calif.) in 500 ml of nitrobenzene at 10 °C were added simultaneously solutions of anhydrous aluminum chloride (21.4 g, 0.16 mol) in 75 ml of nitrobenzene and *p*-anisoyl chloride (13.7 g, 0.086 mol) in 50 ml of nitrobenzene. The mixture was heated to 35 °C for 4 h and cooled to 10 °C. HCl (4 N, 250 ml) was added, and the mixture was then heated to 60 °C, diluted with 250 ml of water, and stirred for an additional 5 min. The solid was recovered by suction filtration. The resin was then washed with tetrahydrofuran (500 ml), tetrahydrofuran–water (1:1 v/v) (500 ml), water (500 ml), water–methanol (1:1 v/v) (500 ml), and methanol (500 ml) and dried in vacuo at 45 °C. Recovery was 58.5 g; the product exhibited a strong carbonyl absorption at 1675 cm^{-1} (KBr pellet).

Anisylformylaminomethylpolystyrene–1% Divinylbenzene (Leuckart Reaction). The *p*-anisoylated Bio-Beads (58.5 g), ammonium formate (165 g), formamide (200 ml), 88% formic acid (250 ml), and nitrobenzene (600 ml) were added to a 2-l., three-necked, round-bottomed flask, fitted with a mechanical stirrer, thermometer, and a Dean-Stark recovery trap. The reaction mixture was heated to 165–175 °C while stirring. After 2 h 50 ml of 88% formic acid was added and another 50 ml of 88% formic acid every hour. After a total of 5 h the reaction mixture was cooled to room temperature and the solid isolated by filtration, washed with tetrahydrofuran (750 ml), tetrahydrofuran–ethanol (1:1 v/v) (750 ml), and ethanol (750 ml).

Anisylaminomethylpolystyrene–1% Divinylbenzene. The Leuckart reaction product, still wet with ethanol, was suspended in 400 ml of ethanol. Concentrated HCl (175 ml) was added and the mixture was stirred under reflux for 2 h. After cooling to room temperature, the solid was collected by filtration and washed by suspension for several hours in ethanol (4 × 250 ml) until the wash gave a negative silver chloride test. The polymer was dried to a constant weight (59.9 g). The *p*-methoxybenzhydrylamine resin contains 0.87% nitrogen, which is equivalent to 0.62 mequiv amine/g resin.

Determination of Secondary Amine Content of *p*-Methoxybenzhydrylamine Resin (Demjanov Reaction). One gram of the above described resin was suspended in 200 ml of *p*-dioxane and 2 N HCl (60 ml) was added with stirring over 30 min. Sodium nitrite (0.69 g, 0.01 mol) in water (20 ml) was added and the reaction mixture was then stirred at room temperature for 2 h. The product was recovered by filtration and washed with chloroform (50 ml) and methanol (150

ml), and then air dried. The resin was ninhydrin negative (31). Elemental analysis for nitrogen gave a value of 0.07% N, which corresponds to 0.02 mequiv of secondary amine/g resin.

Comparison of Leuckart Reaction vs. Oxime Reduction in the Preparation of Benzhydrylamine Resins. Benzoylated copolystyrene–1% divinylbenzene was prepared as described for the *p*-anisoylated resin. Benzoylated resin (15g) was converted to the oxime by the method described by Pietta et al.[9] Two aliquots of the resulting oxime were then reduced with bis(2-methoxyethoxy)aluminum hydride (Vitride) in benzene using two modes of addition: (1) addition of oxime resin to a stirred excess Vitride solution in benzene, and (2) addition of Vitride solution to a stirred suspension of excess oxime resin in benzene. The third aliquot of benzoylated resin was converted to benzhydrylamine resin by the Leuckart reaction described above. The resulting products were analyzed for total amine content, subjected to the Demjanov reaction, and analyzed for secondary amine content (Table I).

Preparation of Dipeptides on *p*-Methoxybenzhydrylamine Resin. In a 100-ml solid-phase reaction vessel (Schwarz-Mann), 3 g of *p*-methoxybenzhydrylamine hydrochloride resin (0.62 mequiv amine/g polymer) was shaken with 35 ml of 10% v/v *N,N*-diisopropylethylamine in CH_2Cl_2 for 10 min. After removal of the base the resin was treated with 4.66 mequiv of the appropriate *tert*-butyloxycarbonyl amino acid (2.5-fold excess) in 20 ml of CH_2Cl_2 and 0.96 g (4.66 mequiv) of DCCI in 20 ml of the same solvent for 40 min at room temperature. The resin was washed with 35-ml portions each of 25% acetic acid in CH_2Cl_2, twice with $CHCl_3$, and twice with CH_2Cl_2. In the instances when the ninhydrin test was positive, the coupling was repeated using a 1.16-fold excess. In case of a negative ninhydrin test a 150-mg sample was withdrawn for amino acid analysis.

The Boc protecting group was removed by treatment of the resin with 35 ml of 33% (v/v) trifluoroacetic acid–methylene chloride for 45 min at room temperature. After treatment with two 35-ml portions of *N,N*-diisopropylethylamine–CH_2Cl_2 (8% by volume) for 10 min, the resin was washed with 35-ml portions each of $CHCl_3$ and CH_2Cl_2. The deprotected resin was treated with *tert*-butyloxycarbonylalanine (0.88 g, 4.66 mequiv) in 20 ml of CH_2Cl_2 and DCCI (0.96 g, 4.66 mequiv) in 20 ml of the same solvent for 40 min at room temperature. The resin was then washed as previously described, a small sample subjected to the ninhydrin test,[31] and recoupling was performed if necessary. A sample of dipeptide-substituted resin was hydrolyzed in 1:1 propionic acid–concentrated HCl at 135 °C for 4 h[25] and analyzed for amino acid content (see Table II).

Cleavage with HF of Alanylphenylalanine Amide from *p*-Methoxybenzhydrylamine Resin. Boc-Ala-Phe-resin (1.25 g) was placed in a Teflon cleavage vessel, 6.5 ml of anisole was added, and 20 ml of anhydrous HF was collected. The mixture was stirred for 45 min at room temperature. The HF was removed under reduced pressure and the resin dried in vacuo to remove the anisole. The dipeptide was extracted with 4% aqueous acetic acid (50 ml). Lyophilization of the extract yielded 111 mg (92%) of dipeptide amide hydrogen fluoride salt; TLC (*n*-BuOH–AcOH–H_2O, 4:1:1) shows one spot, R_f 0.37 as revealed by chloride tolidine reagent.[32] Anal. Calcd for $C_{12}H_{18}N_3O_2F \cdot H_2O$: C, 52.7; H, 7.38; N, 15.4. Found: C, 52.5; H, 7.20; N, 15.1. Amino acid analysis after 16 h of hydrolysis in 1:1 propionic acid–concentrated HCl at 135 °C gave the following molar ratios: Phe, 1.0; Ala, 0.98.

Z-L-Pyroglutamyl-N^{im}-tosyl-L-histidyl-L-prolyl-*p*-methoxybenzhydrylamine Resin. Boc-proline-substituted resin (5 g) prepared as described (Table II) was utilized for the preparation of the hormone. The following cycles of deprotection, neutralization, and coupling were carried out for the introduction of each of the other two residues (His, <Glu) in the peptide: (1) cleavage of the Boc group by two successive treatments with 40 ml of 33% trifluoroacetic acid in CH_2Cl_2 for 45 min at room temperature; (2) one wash with 40 ml of CH_2Cl_2; (3) treatment with three 40-ml portions of *N,N*-diisopropylethylamine in CH_2Cl_2 (8% by volume); (4) addition of 7.76 mequiv (2.5-fold excess) of Boc-N^{im}-tosyl-His in 20 ml of CH_2Cl_2 and 5 min of shaking; (5) addition of 7.76 mequiv of DCCI in 20 ml of CH_2Cl_2 followed by overnight reaction period; (6) one wash with 40 ml of 25% acetic acid in CH_2Cl_2; (7) one wash with 40 ml of $CHCl_3$; (8) ninhydrin test (if negative, go to step 9); (9) repetition of steps 1–8, this time coupling Z-<Glu, 7.76 mequiv; (10) one wash with 40 ml of anhydrous ethanol. After air drying 6.37 g of resin tripeptide was recovered.

L-Pyroglutamyl-L-histidyl-L-proline Amide (TRH). Z-<Glu-N^{im}-tosyl-His-Pro-NH_2 (1.29 g) was removed from the resin with HF as described above. The resin–peptide mixture was washed with anhydrous ethyl ether (3 × 25 ml) and the peptide extracted with 40 ml of MeOH. Evaporation of solvent yielded 189 mg of crude tripeptide. The hormone was purified on a silica gel column (13 g, 12 × 2 cm) by elution with MeOH–$CHCl_3$ (3:7 v/v). Yield of TRH was 132 mg (78%). The R_f on TLC was within experimental error when compared with an authentic sample in three different solvent systems: R_f 0.24 (*n*-BuOH–AcOH–EtOAc–H_2O, 1:1:1:1); R_f 0.63 ($CHCl_3$–MeOH–NH_4OH, 6:4.5:2); and R_f 0.40 (EtOH–H_2O, 7:3); $[\alpha]^{24}D$ −40° (*c* 1, MeOH). Amino acid analysis after 24 h of hydrolysis with 6 N HCl gave the following molar ratios: Pro, 1.0; Glu, 1.0; His, 0.99; and NH_3, 0.95.

Boc-L-Isoleucyl-L-glutaminyl-L-asparaginyl-*p*-methoxybenzyhydrylamine Resin. The *p*-methoxybenzhydrylamine hydrochloride resin (5 g) was shaken with 45 ml of 10% by volume *N,N*-diisopropylethylamine in CH_2Cl_2 for 10 min. After removal of the base the resin was treated with 4.6 g (fourfold excess) of Boc-Asn-ONp and 0.4 g of *N*-hydroxybenzotriazole in 75 ml of *N*-methyl-2-pyrrolidone for 16 h. The resin was washed with 70-ml portions each of CH_2Cl_2, absolute ethanol, 25% acetic acid in CH_2Cl_2, and CH_2Cl_2. The Boc protecting group was removed by treatment with 70 ml of 33% by volume trifluoroacetic acid in CH_2Cl_2 for 45 min at 23 °C. After treatment with two 70-ml portions of *N,N*-diisopropylethylamine–CH_2Cl_2 (8% by volume) for 10 min the resin was washed with two 70-ml portions of CH_2Cl_2. Next the peptide chain was elongated using Boc-Gln-ONp as acylating agent under the conditions described above. After 16 h the resin dipeptide gave a faint positive ninhydrin test. Hence, the resin dipeptide was acylated with acetic anhydride (1 ml) in the presence of *N,N*-diisopropylethylamine (1 ml) in *N*-methyl-2-pyrrolidone (75 ml) for 60 min. After acylation the resin dipeptide gave a negative ninhydrin test. The resin dipeptide was then carried through a deprotection and base treatment cycle. The following cycles were carried out for the coupling of Boc-Ile (at a 2.5-fold excess): a solution of Boc-Ile (7.75 mequiv, 1.86 g) and *N*-hydroxybenzotriazole (1.57 g, 11.6 mequiv) in 50 ml of *N*-methyl-2-pyrrolidone was added to the resin dipeptide and shaken for 10 min. At this point a solution of DCCI (1.60 g, 7.75 mequiv) in 20 ml of CH_2Cl_2 was added to the mixture while shaking was continued for 4 h. Since the ninhydrin test was positive a second coupling was carried out using Boc-Ile (1.20 g, 5 mequiv) and *N*-hydroxybenzotriazole (1 g, 7.5 mequiv) in 50 ml of *N*-methyl-2-pyrrolidone followed by DCCI (1 g, 5 mequiv) as described. Shaking continued for 4 h until the ninhydrin test was negative. The tripeptide resin was washed with 70-ml portions of *N*-methyl-2-pyrrolidone, $CHCl_3$, anhydrous ethanol, and twice with CH_2Cl_2. The resin tripeptide was then dried in vacuo (5.95 g, 93%).

L-Isoleucyl-L-glutaminyl-L-asparaginamide·HF. Boc-Ile-Gln-Asn-resin (1.3 g) was placed in a Teflon cleavage vessel and 4 ml of anisole was added. Treatment with 20 ml of anhydrous HF for 45 min with stirring at 0 °C liberated the peptide amide which was isolated as described above. Yield of crude product was 240 mg (94% based on the substitution level of the starting resin). Crystallization from water/2-propanol gave 164 mg (65%): R_f 0.19 (*n*-BuOH–AcOH–H_2O, 3:1:1); R_f 0.42 (*n*-BuOH–AcOH–Pyr–H_2O, 15:3:10:6); R_f 0.35 (EtOH–H_2O, 7:3); $[\alpha]^{23}D$ −40° (*c* 0.65, 1 N AcOH).

Amino acid analysis of a sample hydrolyzed for 22 h with 6 N HCl gave the following molar ratios: Asp, 1.0; Glu, 1.0; Ile, 0.93; and NH_3, 2.8.

Boc-L-Isoleucyl-L-glutaminyl-L-asparaginamide. Amidation of Boc-Ile-Gln-Asn-OBzl (375 mg, 0.67 mequiv) gave 300 mg (94.5%) of product: mp 232–234 °C; $[\alpha]^{24}D$ −10° (*c* 1, AcOH); R_f 0.39 (*n*-BuOH–AcOH–H_2O, 4:1:1). Anal. Calcd for $C_{20}H_{36}N_6O_7 \cdot \frac{1}{2}MeOH$: C, 50.4; H, 7.84; N, 17.2. Found: C, 50.6; H, 7.98; N, 17.1.

L-Isoleucyl-L-glutaminyl-L-asparaginamide HF. Boc-Ile-Gln-Asn-NH_2 (250 mg, 0.53 mequiv) was suspended in 2 ml of pure anisole and subjected to HF deprotection for 10 min at 0 °C by the method described above. After evaporation of the HF-anisole, the residue was dissolved in 20 ml of water, the aqueous solution washed with ethyl ether (2 × 20 ml), and the product recrystallized from water/2-propanol (190 mg, 91.9%): R_f 0.19 (*n*-BuOH–AcOH–H_2O, 3:1:1); R_f 0.42 (*n*-BuOH–AcOH–Pyr–H_2O, 15:3:10:6); R_f 0.35 (EtOH–H_2O, 7:3); $[\alpha]^{24}D$ −41° (*c* 0.75, 1 N AcOH). Amino acid analysis of a sample hydrolyzed for 22 h with 6 N HCl gave the following molar ratios: Asp, 1.0; Glu, 1.0; Ile, 0.98; and NH_3, 3.0.

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Registry No.—Copolystyrene–divinylbenzene, 9003-70-7; *p*-anisoyl chloride, 100-07-2; anisylformylaminomethane, 3400-22-4; anisylaminomethane, 5961-59-1; *p*-methoxybenzhydrylamine, 2538-34-3; *p*-methoxybenzhydrylamine HCl, 5267-46-9; *tert*-butyloxycarbonylalanine, 15761-38-3; alanylphenylalaninamide HF, 60195-80-4; Boc-Ala-Phe, 2448-58-0; Z-L-pyroglutamyl-N^{im}-tosyl-L-histidyl-L-prolyl, 60195-81-5; Boc-proline, 15761-39-4; Boc-N^{im}-tosyl-His, 35899-43-5; Z-<Glu, 32159-21-0; L-pyroglutamyl-L-histidyl-L-proline amide, 24305-27-9; Z-<Glu-N^{im}-tosyl-His-Pro-NH_2, 35899-45-7; Boc-L-isoleucyl-L-glutaminyl-L-asparagine, 52574-14-8; Boc-Asn-ONp, 4587-33-1; Boc-Gln-ONp, 15387-45-8; Boc-Gln-Asn-ONp, 60195-82-6; Boc-Ile, 13139-16-7; L-isoleucyl-L-glutaminyl-L-asparaginamide HF, 60195-83-7; Boc-L-isoleucyl-L-glutaminyl-L-asparaginamide, 60209-57-6; Boc-Ile-Gln-Asn-O-Bzl, 60209-58-7; Boc-Gly, 4530-20-5; Boc-Phe, 13734-34-4; Boc-Val, 13734-41-3; Boc-Glu(OBzl), 30924-93-7; Boc-Ala-Gly, 28782-78-7; Boc-Ala-Pro, 33300-72-0; Boc-Ala-Phe, 2448-58-0; Boc-Ala-Val, 60209-59-8; Boc-Ala-Glu(OBzl), 60209-60-1.

References and Notes

(1) This work was supported in part by USPHS Grant AM-18399. Nomenclature follows the tentative rules of the IUPAC-IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972); **250**, 3215 (1975). Additional abbreviations used: Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DCCI, dicyclohexylcarbodiimide; ONp, *p*-nitrophenyl ester.
(2) P. G. Pietta and G. R. Marshall, *Chem. Commun.*, 650 (1970).
(3) G. R. Marshall and R. B. Merrifield, "Biochemical Aspects of Reactions on Solid Supports", G. Stark, Ed., Academic Press, New York, N.Y., 1971.
(4) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(5) M. Bodanszky and J. T. Sheehan, *Chem. Ind. (London)*, 1597 (1966).
(6) W. Kessler and B. Iselin, *Helv. Chim. Acta*, **49**, 1330 (1966).
(7) H. C. Beyerman, H. Hindriks, and E. W. B. DeLeer, *Chem. Commun.*, 1668 (1968).
(8) S. Visser, J. Rocloffs, K. E. T. Kerling, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **87**, 559 (1971).
(9) P. G. Pietta, P. F. Cavallo, K. Takahashi, and G. R. Marshall, *J. Org. Chem.*, **39**, 44 (1974).
(10) P. Rivaille, A. Robinson, M. Kamen, and G. Milhaud, *Helv. Chim. Acta*, **54**, 2772 (1971).
(11) J. Rivier, M. Monahan, W. Vale, G. Grant, and M. Amoss, *Chimia*, **26**, 300 (1972).
(12) W. Vale, G. Grant, J. Rivier, M. Monaham, M. Amoss, R. Blackwell, R. Burgus, and R. Guillemin, *Science*, **176**, 933 (1972).
(13) P. Rivaille and G. Milhaud, *Helv. Chim. Acta*, **55**, 1617 (1972).
(14) M. W. Monahan and J. Rivier, *Biochem. Biophys. Res. Commun.*, **48**, 1100 (1972).
(15) V. J. Hruby, F. Muscio, C. M. Groginsky, P. M. Gitu, D. Saba, and W. Y. Chan, *J. Med. Chem.*, **16**, 624 (1973).
(16) D. A. Ontjes, J. C. Roberts, J. F. Hennessy, H. J. Burford, and C. W. Cooper, *Endocrinology*, **92**, 1780 (1973).
(17) R. L. Colescott, private communication.
(18) P. G. Pietta, P. F. Cavallo, G. R. Marshall, and M. Pace, *Gazz. Chim. Ital.*, **103**, 483 (1973).
(19) D. R. Smith, M. Maienthall, and J. Tipton, *J. Org. Chem.*, **17**, 294 (1952).
(20) M. N. Renick, C. H. Trothier, R. A. Dargnault, and J. D. Defoe, *Tetrahedron Lett.*, 629 (1963).
(21) V. J. Hruby, F. A. Muscio, W. Brauer, and P. M. Gitu in "Chemistry and Biology of Peptides", J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, p 331.
(22) P. A. S. Smith and D. R. Baer, *Org. React.*, **11**, 157–188 (1960).
(23) H. K. Hall, *J. Org. Chem.*, **29**, 3139 (1964).
(24) S. H. Graham and A. j. S. Williams, *Tetrahedron*, **21**, 3263 (1965).
(25) F. C. Westall, J. Scotchler, and A. D. Robinson, *J. Org. Chem.*, **37**, 3363 (1972).
(26) K. Folkers, F. Enzmann, J. Boler, C. Y. Bowers, and A. V. Schally, *Biochem. Biophys. Res. Commun.*, **37**, 123 (1969).
(27) H. Sievertsson, S. Castensson, K. Andersson, S. Bjorkman, and C. Y. Bowers, *Biochem. Biophys. Res. Commun.*, **66**, 3–4, 1401 (1975).
(28) M. E. Celis, S. H. Nakagawa, and R. Walter in "Peptides: Chemistry, Structure, Biology", R. Walter and J. Meienhofer, Ed., Ann Arbor Science Publishers, Ann Arbor, Mich., 1975, p 771.
(29) M. E. Celis, S. Taleisnik, and R. Walter, *Biochem. Biophys. Res. Commun.*, **45**, 564 (1971).
(30) We thank Dr. S. Hase Nakagawa for the preparation of Boc-Ile-Gln-Asn-OBzl synthesized via two successive active ester condensation reactions[33] starting with Asn-OBzl in an overall yield of 40%, mp 211–212 °C dec. Anal. Calcd for $C_{27}H_{41}N_5O_8$: C, 57.5; H, 7.33; N, 12.4. Found: C, 57.4; H, 7.29; N, 12.2.
(31) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(32) H. Zahn and E. Rexroth, *Z. Anal. Chem.*, **148**, 181 (1955).
(33) M. Bodanszky, *Nature (London)*, **175**, 685 (1955).

Synthetic Approaches to 10-Epieudesmane Sesquiterpenes. A Synthesis of Intermedeol

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A number of synthetic approaches to the biosynthetically important 10-epieudesmane sesquiterpene intermedeol (1) have been explored. Reduction of epi-α-cyperone (**6**) with lithium–ammonia and oxidation of the intermediate enolate gave acetoxy ketone **9** as the major product. However, **9** could not be deoxygenated to intermedeol. Reduction of **6** with lithium in ammonia followed by trapping of the derived enolate with diethyl chlorophosphate gave enol phosphate ester **11**. Hydrogenolysis of **11** gave a mixture of three hydrocarbons, **3**, **4**, and **12**, while hydrogenolysis of the related enol phosphate **13** gave only one olefin (**15**) on reduction. The synthesis of **1** from α-agarofuran (**18**) was accomplished by the sequence conversion to the 3,4-oxide (**19**), reduction of **19** to 4β-hydroxydihydroagarofuran (**20**), and lithium–ethylenediamine reduction of **20** to diol **23**, which on partial dehydration afforded intermedeol (**1**). The C-4 epimer of intermedeol, **5**, was synthesized from 10-epieudesma-4,11-diene (**3**) by epoxidation to **24**, dissolving metal reduction of which gave **5**. The structure of **24** was confirmed by its conversion to 4α-hydroxydihydroagarofuran and that of **5** by reduction to the dihydro compound (**26**), which was synthesized by an alternate route.

In the generally accepted biosynthetic scheme[1] for the nonisoprenoid nootkatone–valencene group of sesquiterpenes, 10-epieudesmanes, such as intermedeol (**1**),[2] 10-epi-γ-eudesmol (10-epieudesm-4-en-11-ol, **2**),[3] and the isomeric 10-epieudesmadienes (**3** and **4**)[4] play a key role. These compounds can all, at least in principle, undergo the carbonium ion type rearrangement suggested many years ago by Robinson for the biosynthesis of eremophilone from a eudesmane precursor.[5] In addition, at the time when this work was initiated, an additional sesquiterpenoid alcohol, "paradisiol" (**5**), the C-4 epimer of intermedeol, had been reported.[6] However, the infrared spectrum of "paradisiol" and intermedeol indicated that these compounds were identical, and subsequent synthetic and NMR studies showed that intermedeol was correctly represented by structure **1**,[2c] as originally suggested by Zalkow.[2a] Subsequent direct comparison showed that these compounds are in fact identical.[7]

The obvious starting point for a synthesis of either inter-