Esterification of Neutral and Basic Dialkyl(arylmethylene)sulfonium Resins with N-Protected Amino Acids for Use in Solid Phase Peptide Synthesis[1]

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The esterification step—attachment of a N-protected amino acid *via* a benzyl ester linkage to a solid resin support—of the Merrifield solid phase peptide synthesis has been improved significantly through the use of neutral (**2** and **4**) and, particularly, basic (**5**) dialkyl(arylmethylene)sulfonium resins. When **5** is mixed with a dioxane solution containing an equivalent amount or excess of a N-protected amino acid the neutralization reaction which follows effects an irreversible ion exchange of the resin's bicarbonate anion by the acid's carboxylate anion. On drying the mixture *in vacuo* at 25–35° and subsequent heating, dry or in a solvent of low dielectric constant, at 80–85° for 4–5 hr the ensuing displacement reaction proceeds essentially to completion forming resin esters (**6**) in high yield, generally 87–96%; small amounts (4–13%) of alkyl esters (**7**), easily removed from the resin, are also formed. When R, R of **5** = tetramethylene, a mixture of benzyl (~65–80%) and δ-thiobutyl (~20–35%) esters are formed, both of which are attached to the resin.

Since its inception by Merrifield[2] in 1962 the solid phase synthesis of peptides has achieved substantial acceptance as a method for synthesizing peptides. Much of the research reported by Merrifield and others in this area has dealt with the syntheses of various peptides[3-12] including the A and B chains of insulin[7] and improvements in the methodology of the technique.[12-19] For the most part improvements in the methodology have involved the coupling step, *e.g.*, use of N-ethyl-5-phenylisoxazolium-3-sulfonate as a coupling agent for N-protected amino acids having side-chain amide or hydroxyl functions,[18] the termination step, *e.g.*, the application of anhydrous hydrogen fluoride for removal of the completed peptide from its polymer support,[13] and design of reaction apparatus.[16,17] Relatively speaking, little work has been reported[20] on improvement of the first step of the solid phase peptide synthesis, *i.e.*, attachment of the C-terminal N-protected amino acid residue to a polymer support *via* an ester linkage. This is surprising and ironic since the esterification step is one of the poorer aspects of the solid phase peptide synthesis from the standpoint of yield and reaction conditions. In spite of the modified esterification scheme of Bodanszky and Sheehan[20] the Merrifield procedure (Scheme I) still appears to be the method of choice although ester yields are low to moderate.

Therefore, in view of the importance of the esterification step to solid phase peptide synthesis and its present status we undertook a study to find ways of improving it.

Results

We started with partially chloromethylated styrene–divinylbenzene copolymer resin as did Merrifield as well as Bodanszky and Sheehan. It was reasoned that an esterification at a resin's surface or in its matrix by displacement of a group X from a benzylic carbon by a N-protected amino acid carboxylate anion could be facilitated by (a) making X a better leaving group, (b) increasing the swelling of the resin during the reaction to make $-CH_2X$ more accessible, and (c) a combination of these. Proceeding with this view, resins were prepared which contained the more reactive alkylsulfonium group (**2**, X = $-S^+Me_2Cl^-$), a resin swelling alkylammonium group in conjunction with a chloromethyl group (**3**, X = $-N^+Me_3Cl^-$ and Cl), and a combination of alkylsulfonium and alkylammonium groups (**4**) (Scheme II). These resins were subjected to the Merrifield esterification conditions with N-*t*-butyloxycarbonylglycine and N-*t*-butyloxycarbony-L-valine. The esterification results are summarized in Table I.

During the course of this work our attention was directed to another technique[21] of utilizing sulfonium compounds for preparing esters. This technique involves conversion of the neutral sulfonium compound to a basic sulfonium compound by exchange of its chloride (or other ion) anion with a basic anion such as bicarbonate and hydroxide ions. When the basic sulfonium compound is brought into contact with a carboxylic acid the neutralization reaction which results thereby effects an irreversible ion exchange of the basic anion by the corresponding carboxylate anion of the acid such that on drying the sulfonium and carboxylate functions are left as the only opposing ions, *i.e.*, nucleophile and electrophile, and on heating displacement of the sulfonium group occurs and an ester is formed.

(1) Presented at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968.

(2) R. B. Merrifield, *Fed. Proc.*, **21**, 412 (1962).

(3) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).

(4) (a) R. B. Merrifield, *ibid.*, **86**, 304 (1964); (b) R. B. Merrifield, *Biochemistry*, **3**, 1385 (1964).

(5) D. W. Wooley, *J. Amer. Chem. Soc.*, **88**, 2309 (1966).

(6) R. B. Merrifield, *J. Org. Chem.*, **29**, 3100 (1964).

(7) A. Marglin and R. B. Merrifield, *J. Amer. Chem. Soc.*, **88**, 5051 (1966).

(8) V. A. Najjar and R. B. Merrifield, *Biochemistry*, **5**, 3765 (1966).

(9) T. Mizojucki and D. W. Wooley, *J. Med. Chem.*, **10**, 251 (1967).

(10) (a) J. M. Stewart, J. D. Young, E. Benjamini, M. Shimizu, and C. Y. Leung, *Biochemistry*, **5**, 3396 (1966); (b) J. D. Young, E. Benjamin, J. M. Stewart, and C. Y. Leung, *ibid.*, **6**, 1455 (1967).

(11) R. L. Smith, "Synthesis and Microbiological Properties of Some Tetrapeptides and Pentapeptides," University Microfilms, Inc., Ann Arbor, Mich., 1966.

(12) M. C. Kholsa, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 754 (1967).

(13) J. Lenard and A. B. Robinson, *J. Amer. Chem. Soc.*, **89**, 181 (1967).

(14) A. Deer, *Angew. Chem. Intern. Ed. Engl.*, **5**, 1041 (1966).

(15) F. Weygand and U. Ragnarsson, *Z. Naturforsch.*, **21B**, 1141 (1966).

(16) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Science*, **156**, 253 (1967).

(17) R. B. Merrifield, *ibid.*, **150**, 178 (1965).

(18) F. M. Bumpus, M. C. Khosla, and R. R. Smeby, Abstracts, 153rd National Meeting of the American Chemical Society, Miami, Fla., April 1967, p 40M.

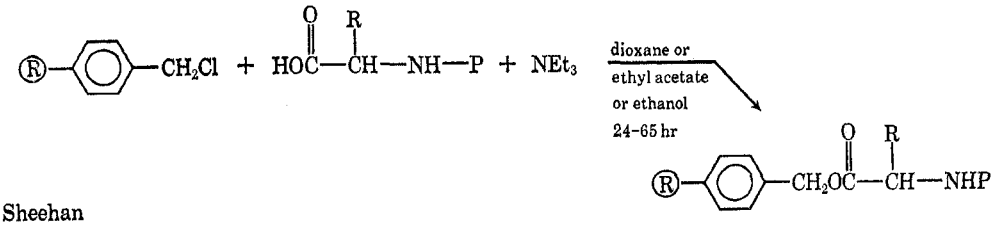
(19) E. P. Semkin, N. D. Gafurova, and L. A. Shchukina, *Khim. Prir. Soedin.*, **3**, 220 (1967).

(20) M. Bodanszky and J. T. Sheehan, *Chem. Ind.* (London), 1597 (1966).

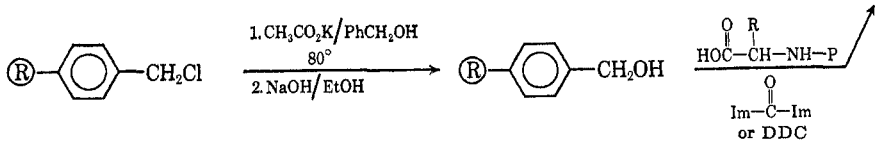
(21) R. A. Wessling, R. G. Zimmerman, J. H. Kerr, and T. Alfrey, Jr., The Dow Chemical Co., unpublished results.

SCHEME I[a]
ESTERIFICATION PROCEDURES

Merrifield

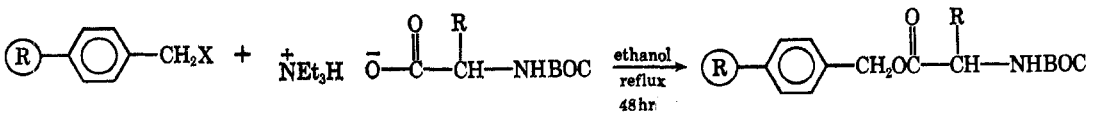


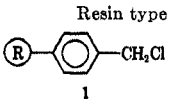
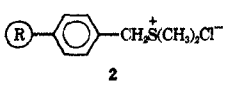
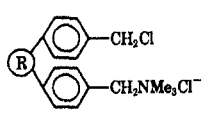
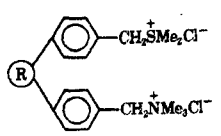
Bodanszky and Sheehan



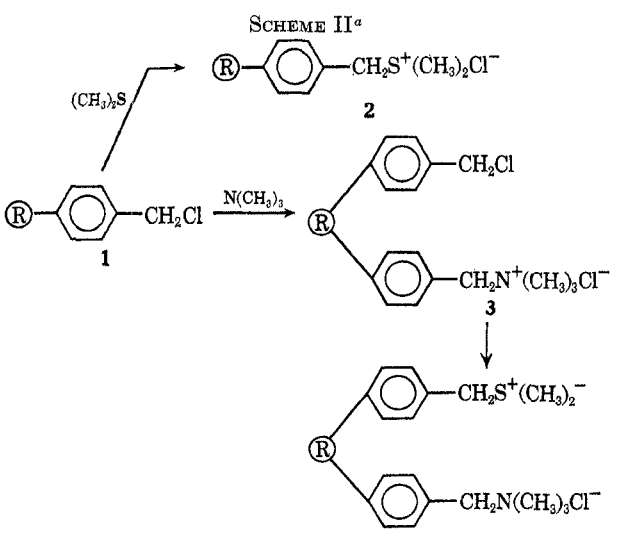
[a] P = protective group, *t*-butyloxycarbonyl (BOC), *o*-nitrophenylsulfenyl (NPS); Ⓡ- = styrene–divinylbenzene copolymer resin; DCC = dicyclohexylcarbodiimide; Im–CO–Im = N,N′-carbonyldiimidazole.

TABLE I
ESTERIFICATION OF BOC-GLYCINE AND BOC-L-VALINE WITH VARIOUS α-SUBSTITUTED ARYLMETHYLENESTYRENE–2% DVB RESINS



Resin type	BOC-amino acid	Functionality X, mmol/g of starting resin: A $-N^+Me_3Cl^-$	B $-Cl$	C $-S^+Me_2Cl^-$	Resin ester product, mmol of amino acid/g	% yield based on: B	C	B + C
 1	Glycine		1.74		0.695	44		
	Valine				0.86	54		
 2	Glycine							
	Valine		0.14	1.14	0.82		73	65
 3	Glycine							
	Valine	1.60	2.47		1.18	57		
 4	Glycine	1.43	0.29	1.74	1.04		61	53
	Valine	1.43	0.29	1.74	1.17		73	63

SCHEME II[a]



[a] This scheme depicts the general mode of preparation of these resins and is not meant to imply that **2**, **3**, and **4** were necessarily derived from the same batch of **1**.

Basic dialkyl- and cycloalkyl(arylmethylene)sulfonium resins were conveniently prepared from the chloride from of the resin by exchange with 1 *M* solutions of potassium bicarbonate or carbonate on a column. Various N-protected amino acids were brought into contact with the basic sulfonium resins in dioxane. After neutralization and drying the resin was heated at 80° for 4–5 hr to effect the esterification (Scheme III). At 45° about 3 days were required to complete the esterification. Completeness of reaction was generally determined by the amount of unchanged amino acid carboxylate anion that could be removed from the resin by washing with 1 *N* HCl in acetic acid. The extent of the side reaction, *i.e.*, formation of N-protected amino acid alkyl ester (**7**, P = BOC), was determined by isolation of the ester by washing the resin with a solvent after the reaction. A number of various modifications of the general procedure were tried (See Experimental Section) and the data are compiled in Table II.

SCHEME III

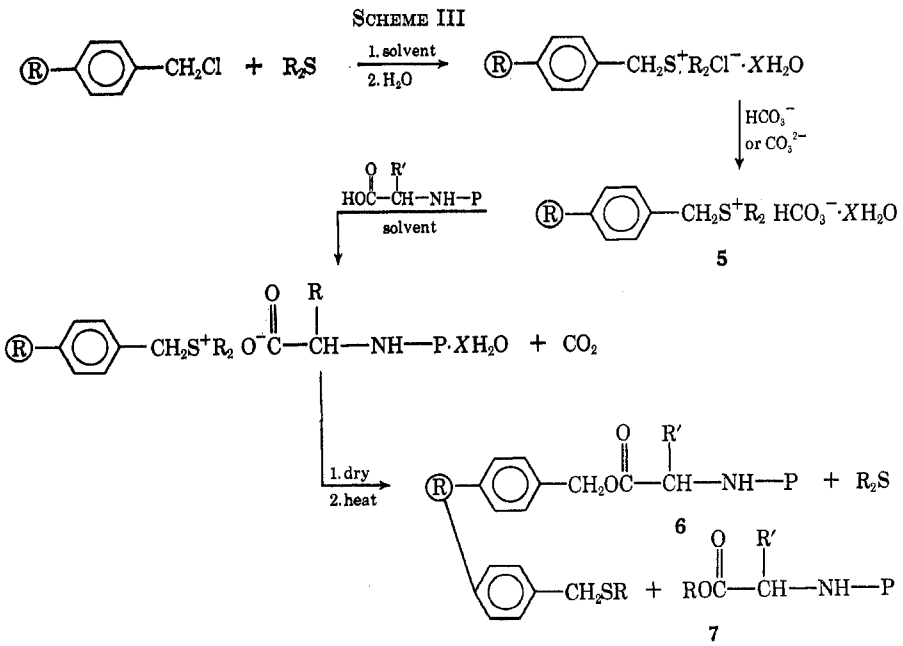
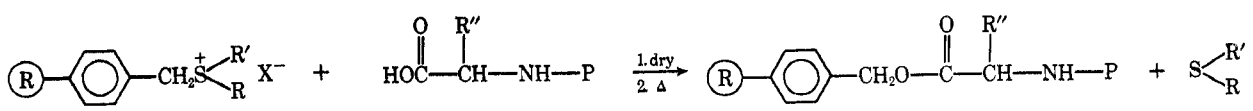
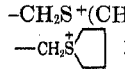


TABLE II[a]

ESTERIFICATION OF N-PROTECTED AMINO ACIDS TO RESIN SUPPORT *via* BASIC SULFONIUM FUNCTIONS



Starting resin[b] sulfonium group	N-Protected amino acid	Ester method (example no.)	% total resin ester conversion	% alkyl ester by-product	Resin ester product: Mmol of amino acid/g of resin ester	Resin ester product: Composition,[c] % Benzyl ester	Resin ester product: Composition,[c] % Butyl ester
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-L-valine	1	95	5	1.00	100	
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-O-benzyl-L-tyrosine	1	92	8	0.832	100	
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-L-proline	2	93	7	2.35	100	
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-L-phenylalanine	2	87	13	2.08	100	
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-L-valine	2	95	5	2.40	100	
	N-BOC-β-benzyl-L-aspartic	3	98		1.41	80[d]	20
	N-BOC-L-valine	3	98		1.61	84[d]	16
	N-BOC-L-proline	3	100		1.60	73[d] (65)[e]	27 (35)
	N-BOC-ε-aminocaproic	4	100		1.58	78[d]	22
	N-NPS-L-glutamine	5			1.21	66	34
$-CH_2S^+(CH_3)_2(CO_3)_{1/2}^{2-}$	N-NPS-L-threonine	6	76		1.22	100	
$-CH_2S^+(CH_3)_2HCO_3^-$	N-BOC-L-valine	7	93	7	1.01	100	
$-CH_2S^+(C_2H_5)_2HCO_3^-$	N-BOC-L-proline	8	96	4	1.43	100	

[a] Abbreviations: P = protective group; BOC = *t*-butyloxycarbonyl; NPS = *o*-nitrophenylsulfenyl; $X^- = HCO_3^-, (CO_3)_{1/2}^{2-}$. [b] Resin, styrene–2% divinylbenzene copolymer resin. [c] Experimental Section (example 3) for definition. [d] By elemental analysis. [e] By cleavage with HBr in trifluoroacetic acid.

Discussion

Perhaps the most serious drawback to the Merrifield resin esterification procedure is the low to moderate yields obtained, *i.e.*, *ca.* 14–50%.[22] In addition the reaction conditions are somewhat exhaustive; hence the likelihood of undesirable side reactions is increased. A milder procedure was desirable.

Bodanszky and Sheehan's procedure does represent an improvement over the Merrifield method with respect to mildness of the N-protected amino acid ester forming step. These are, however, several substantial limitations to this procedure. N-Protected amino containing unprotected hydroxyl groups (*e.g.*, BOC-threonine and BOC-hydroxyproline) cannot be used in this procedure because of the possibility of self-condensation and polymer formation. Side-chain amide functions of glutamine or asparagine may be dehydrated to nitrile functions[20,23,24] by the condensing agents used.

We believe that the esterification procedure employing basic dialkyl(arylmethylene)sulfonium functions has some definite advantages over the other two procedures just described for the attachment of a N-protected amino acid to a resin through an ester linkage. (a) The sulfonium function is a more reactive group than the corresponding chloride function; therefore, it is easier to displace by a nucleophile. (b) By virtue of the basic nature of the resin the N-protected amino acid is converted irreversibly into its

(22) Calculated from the data in ref 3, 4b, 8, 9, and 11.

(23) E. Schröder and K. Lübke, "The Peptides," Vol. I, Academic Press, New York, N. Y., 1965, p 191.

(24) C. R. Marshall and R. B. Merrifield, *Biochemistry*, **4**, 2394 (1965).

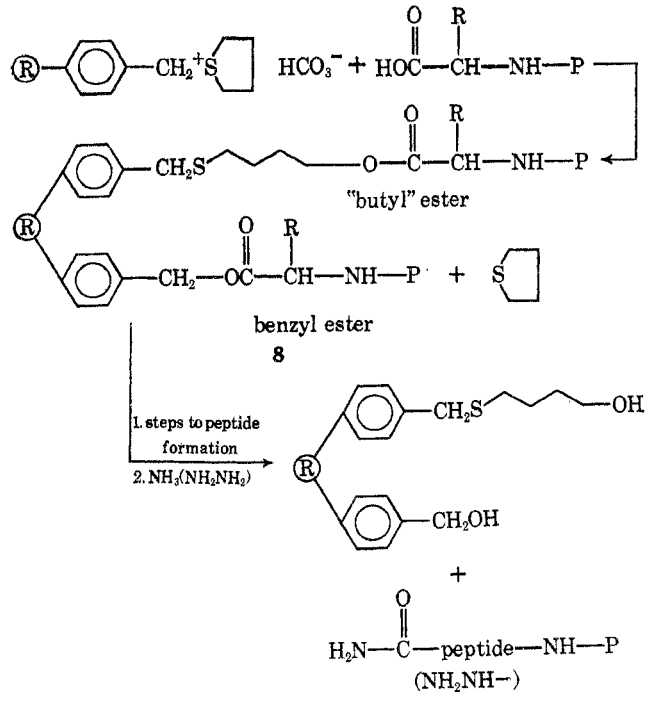
carboxylate anion and simultaneously becomes the resin's anion which after removal of water and solvent from the system becomes the sole nucleophile; thereafter the ensuing displacement reaction is fast and proceeds essentially to completion without the need of a swelling agent. (c) Only a stoichiometric amount of the reacting N-protected amino acid is necessary for the reaction and any unchanged acid can generally be recovered; furthermore, since there is no excess base available or other agents, racemization conditions are diminished. (d) Resins containing higher capacities of covalent attached N-protected amino acids—measured by mmoles of amino acid per gram of resin ester—are readily available by this process (*cf.* the data in Table II). (e) The reaction conditions involved in the esterification process are compatible with N-protected amino acids containing side-chain amide and hydroxyl functions. Rotation measurements of amino acids recovered from resin esters prepared by this procedure indicate that racemization does not occur during the esterification reaction. No D-alloisoleucine formation was detected in the esterification of *t*-BOC-L-isoleucine.

The application of neutral dialkyl(arylmethylene)-sulfonium resins (**2** and **4**) for esterification of N-protected amino acids to resins also appears to offer some advantages over the Merrifield as well as the Bodanszky and Sheehan procedures when all factors are considered. The presence of an alkylammonium function—a resin swelling group—in the resin in addition to the sulfonium function (**4**) does not improve the esterification results over that when only a sulfonium function is in the resin (**2**) presumably the resin swelling capability of the sulfonium function itself is sufficient. Alternately, the resin swelling enhancing feature of the alkylammonium group is offset by the presence of additional chloride ions which compete by mass action phenomena with the N-protected amino acid acyl nucelophile for the sulfonium function.

The preferred dialkyl sulfide for use in preparing the resin sulfonium function is dimethyl sulfide since higher dialkyl sulfides (excepting some cycloalkyl sulfides) react significantly slower.[25] On the other hand, higher dialkylsulfonium derivatives give a smaller amount of alkyl ester by-product (**7**) (*cf.* Table II), but this difference may be significant only in a few cases; *e.g.*, with BOC-proline methyl ester formation occurs to the extent of *ca.* 11% while ethyl ester formation is *ca.* 4%.

The cycloalkyl sulfide, tetrahydrothiophene, reacts on a par with methyl sulfide in the formation of a resin sulfonium derivative. In its ester-forming reactions, a considerable amount of displacement occurs on the tetramethylene side chain resulting in a mixture of resin-attached benzyl and "butyl" esters, the latter ranging from about 20 to 35%. Such a resin ester as **8** would probably find only specialized use in the Merrifield solid phase peptide synthesis because only the benzyl ester portion (*e.g.*, of a completed peptide) would be cleaved by hydrogen bromide, the alkyl "butyl" ester portion[26] being stable to this condition.

SCHEME IV



Such a resin ester, however, might be useful if an amide or a hydrazide of the synthesized peptide was desired since both ester forms would react in the terminal step with ammonia or hydrazine (Scheme IV).[27]

Although it was not of prime interest, *per se*, to prepare resin esters of high capacity, *i.e.*, with a large percentage (~50%) of the aromatic rings substituted, the feasibility of their preparation has been demonstrated by this work. Indeed, it should be noted that the desired extent of ring substitution can be controlled by the extent of chloromethylation of the starting resin.

N-Protected amino acid resin esters prepared by procedures described in this work are currently being used successfully for the solid phase synthesis of various kininlike peptides and these results will be reported elsewhere.

Experimental Section

Melting and boiling points are uncorrected. The N-*t*-butyloxycarbonylamino acids were prepared according to the general procedures of Schwyzer, *et al.*[28] The N-*o*-nitrophenylsulfenylamino acids were prepared as described by Zervas.[29]

The ionic chloride content of a resin (milliequivalents per gram of resin) was determined by suspending a weighed sample in 5 *N* nitric acid and titrating potentiometrically, while stirring, with 0.1 *N* silver nitrate solution. Covalent content of a resin was determined by allowing weighed samples to stand under aqueous 25% trimethylamine solution (at least a ten-fold molar excess used) in a covered beaker for 18–24 hr. The uncovered samples were then warmed on a steam bath for about 1–2 hr longer, cooled, carefully acidified with an excess of 5 *N* nitric acid, and titrated potentiometrically with 0.1 *N* silver nitrate as before. This treatment also gave the total, ionic and covalent, chloride content when both forms were present; thus the covalent chloride content was obtained by determining the ionic and total chloride content and subtracting the former from the latter.

(25) E. B. Trostyankaya, I. P. Loser, and S. B. MaKarova, *Vysokomol. Soedin.*, **5**, 1924 (1963); *Chem. Abstr.*, **60**, 9425 (1964).

(26) Possibly an alkyl ester of this type could be cleaved by anhydrous hydrogen fluoride; see ref 13.

(27) M. Bodanszky and J. T. Sheehan, *Chem. Ind.* (London), 1423 (1964).

(28) R. Schwyzer, P. Sieber, and H. Kappeler, *Helv. Chim. Acta*, **42**, 2622 (1959).

(29) L. Zervas, D. Borovas, and E. Gazier, *J. Amer. Chem. Soc.*, **85**, 3660 (1963).

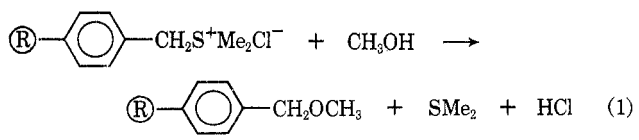
Amino acids were determined in aqueous solutions by the ninhydrin method by S. Webber of the Special Services Laboratory.[30] Infrared analyses of resin samples were recorded and interpreted by R. A. Nyquist of the Chemical-Physics Research Laboratory.[30] Elemental analyses on resin samples were determined by L. Swim of the Special Services Laboratory.[30]

Dimethyl(arylmethylene)sulfoniumstyrene–2% Divinylbenzene Copolymer Resins. A. Bicarbonate Form.—A stoppered suspension of 75 ml each of methanol, methylene chloride, and water, 25 g (113 mmol of $-CH_2Cl$) of chloromethylated styrene–2% divinylbenzene copolymer resin (200–400 mesh; 4.53 mequiv of Cl/g corresponding to about 69% of the benzene rings of the resin chloromethylated), and 13 g (210 mmol) of methyl sulfide was stirred with the aid of a magnetic stirrer for 4.5 days, stirring becoming more difficult as the resin swelled during the reaction. The polymer was collected on a sintered-glass funnel and washed successively with dioxane–water, (3:1) dioxane–1 *N* HCl (3:1), dilute methanol, methanol, and twice with water. After the last wash, the bulk of water was removed gently from the resin and the wet resin was bottled, wt 136 g (approximately 80% water). The ionic and total chloride content of the resin were determined as 0.785 and 0.815 mequiv/g, respectively, making the conversion of chloromethyl functions to sulfonium functions 95%.

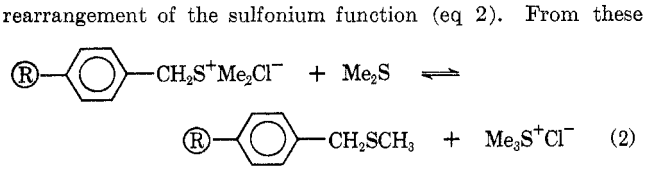
For conversion to the bicarbonate form, 119 g (93.8 mequiv of Cl^-) of the chloride form of the resin was placed in a glass column (0.9 × 12 in.) as a water suspension. The column was drained free of water, care being taken to eliminate channeling. Then 250 ml (250 mequiv) of 1 *N* potassium bicarbonate was passed slowly through the column followed by 150 ml of water. The eluent and wash were collected and titration of an aliquot showed that the total chloride ion exchanged was 93.3 mequiv (99.5%). The resin was removed from the column, collected on a filter and further washed with water until the wash was free of bicarbonate ions. There was obtained 139 g of the bicarbonate form of the resin.

A potentiometric titration of the resin with 0.1 *N* sulfuric acid showed the resin to contain 0.651 ± 0.008 mequiv HCO_3^-/g. The resin was stored wet in a refrigerator.

B. Carbonate Form.—A stoppered suspension of 10 g (33.2 mmol of $-CH_2Cl$) of chloromethylated styrene–2% divinylbenzene copolymer resin (200–400 mesh), 5 ml (68.3 mmol) of methyl sulfide, 50 ml of methanol, and 25 ml of methylene chloride was stirred at room temperature for 5 days. The resin was collected on a sintered-glass funnel and washed with methanol. The mother liquor and wash were combined and retained. The resin was further washed as described in A. Obtained was 48.7 g of wet sulfonium resin. The ionic and total chloride content of the resin were determined as 0.640 and 0.669 mequiv/g, respectively. The combined mother liquor and initial wash (above) were diluted with *ca.* equal volumes each of water and methylene chloride and the resulting layers separated. The organic layer was extracted twice with water and these extracts were combined with the aqueous layer; aliquots were titrated with 0.1 *N* silver nitrate for chloride ion content and with 0.1 *N* potassium hydroxide for hydrogen ion (acid) content. Chloride ion content amounted to a total of 0.20 mequiv, and hydrogen ion content 0.14 mequiv. The acid content is a measure of solvolysis of the sulfonium (and possibly $-CH_2Cl$) function (eq 1). The chloride content is a measure of solvolysis plus



rearrangement of the sulfonium function (eq 2). From these



data, sulfonium conversion, unchanged chloromethyl, solvolysis, and rearrangement were 94, 4.2, 0.4, and 0.2%, respectively.

The chloride form of the sulfonium resin was converted to the carbonate form as described in A for exchange of chloride for bicarbonate; 39.2 g (25.1 mequiv of Cl^-) of resin required 190 ml of 1 *N* potassium carbonate for 95% exchange. There was recovered 29.5 g of light tan resin which contained 0.730 mequiv of CO_3^{2-}/g.

Tetramethylene(arylmethylene)sulfoniumstyrene–2% Divinylbenzene Copolymer Resin. Bicarbonate Form.—A stoppered suspension of 20 g (66.4 mmol of $-CH_2Cl$) of chloromethylated styrene–2% divinylbenzene copolymer resin (200–400 mesh; 3.32 mequiv of Cl/g corresponding to approximately 41% of the aryl groups of the resin chloromethylated) in 50 ml each of methylene chloride and methanol and 12 g (137 mmol) of tetrahydrothiophene was stirred at room temperature. Because of severe swelling of the resin it was necessary to add 25-ml portions of methanol after the first and third days of the reaction to facilitate stirring. At the end of 6 days, the resin was collected on a sintered-glass funnel and washed well with methanol, the supernatant liquor and methanol washes being collected together and retained. The resin was further washed as described in the preceding example, wet wt 87.5 g. The ionic and total chloride content were 0.685 and 0.723 mequiv/g, respectively. The acid content in the supernatant liquor and wash (above) was determined by titration with standard KOH and amounted to 2.2 mequiv. Therefore, the values for sulfonium conversion, solvolysis [of either $-CH_2Cl$ or $-CH_2S^+(CH_2)_4$], and unchanged chloromethyl groups were 91.6, 3.4, and 5%, respectively.

Conversion of 66.8 g (45.8 mequiv) of the chloride form to the bicarbonate form of the resin was accomplished as described in the preceding experiment using 150 ml (150 mequiv) of 1 *N* potassium bicarbonate. The resin contained 0.578 mequiv/g.

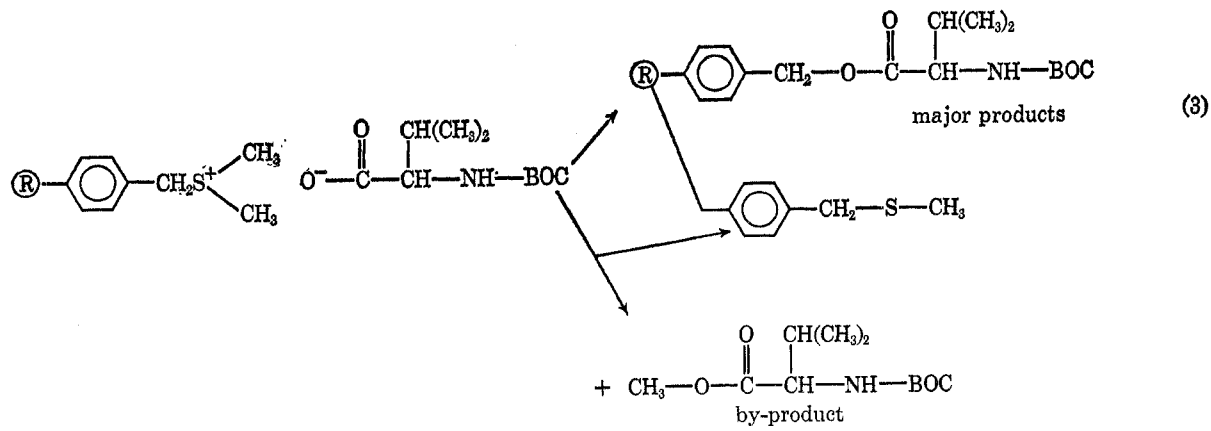
Esterification Reactions of N-Protected Amino Acids and Basic Dialkyl(arylmethylene)sulfoniumstyrene–2% Divinylbenzene Copolymer Resins. Example 1.—To a suspension of 3.80 g (2.12 mequiv of HCO_3^-) of dimethyl(arylmethylene)sulfoniumstyrene–2% divinylbenzene copolymer resin bicarbonate form in 15 ml of dioxane was added a solution of 0.502 g (2.31 mmol) of N-*t*-butyloxycarbonyl-L-valine in 10 ml of dioxane. The resulting mixture was thoroughly stirred as carbon dioxide was evolved. After standing for about 15 min the resin mixture was filtered under suction through a tared sintered-glass funnel and washed three to four times with 5–10 ml of dioxane. [The mother liquor filtrate and wash were retained and upon evaporation left 0.042 g (0.19 mmol) of unchanged acid, the infrared spectrum of which was identical with that of the starting N-*t*-butyloxycarbonyl-L-valine.] The resin, 5.595 g, was dried (in funnel) *in vacuo* over solid KOH with the aid of a mechanical pump at 25–35° for 3 hr, dry wt 2.442 g. The resin was then heated *in vacuo* at 80° for 3.75 hr to complete the esterification reaction, resin wt 2.021 g. The resin product was then swelled and washed with methylene chloride to remove by-product N-*t*-butyloxycarbonyl-L-valine methyl ester (eq 3).

Evaporation of the combined methylene chloride washes left 0.024 g (0.10 mmol) of methyl ester. Identification of the methyl ester was based on infrared data [ν (Nujol) 3385 (NH), 1760 (ester >C=O), 1735 cm^{-1} (urethan >C=O)] and conversion to L-valine methyl ester hydrochloride in methanolic HCl; mp 164–167°, on admixture with authentic L-valine methyl ester hydrochloride (mp 168–169°), was not depressed. Treatment of a 0.245-g sample of the resin product with 1 *N* HCl in acetic acid (as described below) showed the esterification of the acid to the resin to be complete.

The net amount of N-*t*-butyloxycarbonyl-L-valine used was 2.12 mmol of which 0.10 mmol (5%) was converted to the methyl ester and 2.02 mmol (95%) was attached to the resin (benzyl ester). Therefore, the mmoles of N-*t*-butyloxycarbonyl-L-valine per gram of resin = 1.00.

A sample of N-*t*-butyloxycarbonyl-L-valine resin ester (2.40 mmol of BOC-L-valine/g resin of ester, prepared according to the procedure in example 2) was suspended in trifluoroacetic acid and anhydrous HBr was bubbled through the suspension for 1.5 hr to decouple the amino acid from the resin. After filtration of the mixture and evaporation of the filtrate the crude valine hydrobromide remaining was dissolved in water and put through a Dowex 44 (acetate form) column. The valine obtained by evaporation of the eluate was further purified on a Dowex 50X4 (200–400 mesh) column, 1.25 × 37 cm, equilibrated at pH 4 with 0.1 *N* pyridine acetate buffer. For comparison a sample of N-*t*-butyloxycarbonyl-L-valine, which was used to make the resin ester, was "decoupled" and purified in an analogous manner. Rotation values for valine recovered from the resin ester and the

(30) The Dow Chemical Co.



(3)

control were $[\alpha]^{25}D$ +25.5° (*c*, 0.76, 6 *N* HCl) and $[\alpha]^{25}D$ +25.7° (*c*, 0.64, 6 *N* HCl), respectively, showing, within experimental error, that no racemization had occurred during the esterification. The rotation value for L-valine used to prepare the N-*t*-butyloxycarbonyl-L-valine was $[\alpha]^{25}D$ +26.7° (*c*, 0.71, 6 *N* HCl) indicating that a little racemization had occurred during the preparation of the N-*t*-butyloxycarbonyl-L-valine.

Example 2.—A solution of 1.546 g (7.18 mmol) of N-*t*-butyloxycarbonyl-L-proline in 20 ml of dioxane was poured onto 10.63 g (6.92 mequiv of HCO_3^-) of dimethyl(arylmethylene)sulfoniumstyrene-2% divinylbenzene copolymer resin bicarbonate form in a tube and the mixture was stirred as carbon dioxide was evolved. Additional dioxane was used to bring all of the resin in contact with the acid's solution. The mixture was filtered (recovered 0.110 g, 0.51 mmol of unchanged acid) and the resin (6.90 g) was dried for 2 hr as described in example 1. The resin (3.82 g) was heated *in vacuo* as before at 80° for 4 hr, air cooled (wt 2.735 g), swelled, and washed well with methylene chloride to remove N-*t*-butyloxycarbonyl-L-proline methyl ester [ν (Nujol) 1765 (ester >C=O), 1700 cm^{-1} (urethan >C=O)], 0.108 g (0.47 mmol). The resin was finally dried at 45° *in vacuo* for 2 hr, wt 2.639 g.

Examination of a 0.211-g sample of the resin product with 1 *N* HCl in acetic acid (see below) showed the esterification reaction to be complete.

The net amount of N-*t*-butyloxycarbonyl-L-proline used was 6.67 mmol of which 0.47 mmol (7%) was converted into methyl ester and 6.20 mmol (93%) was attached to resin as benzyl ester; the mmoles of N-*t*-butyloxycarbonyl-L-proline per gram of resin = 2.35.

Example 3.—A solution of 0.474 g (1.47 mmol) of N-*t*-butyloxycarbonyl-β-benzyl-L-aspartic acid in 8 ml of dioxane was added to 2.35 g (1.36 mequiv of HCO_3^-) of tetramethylene(arylmethylene)sulfoniumstyrene-2% divinylbenzene copolymer resin bicarbonate form in a test tube (12 × 125 mm). The mixture was stirred and the rest of the esterification procedure was carried out as described in example 2. There was recovered 0.049 g (0.15 mmol) of unchanged acid making the net amount of acid reacting 1.32 mmol. The resin weight after heating was 0.935 g; therefore, the mmoles of N-*t*-butyloxycarbonyl-β-benzyl-L-aspartic acid per gram of resin = 1.41 (1.43 based on N found, 2.0%).

Treatment of a 0.165-g sample of the resin product with 1 *N* HCl in acetic acid as described below showed the esterification reaction to be 98% complete.

To estimate the proportion of benzyl and butyl ester content of the resin arising from the course of the displacement reaction, the resin was washed with methylene chloride, dried, and the nitrogen and sulfur content was determined. (Found: N, 2.0; S, 0.9.) This corresponds to *ca.* 80% benzyl ester and 20% butyl ester based on the relative gram-atoms of N and S found and their distribution in each ester type.

Example 4.[31]—To 2.53 g (1.47 mequiv of HCO_3^-) of tetramethylene(arylmethylene)sulfoniumstyrene-2% divinylbenzene copolymer resin bicarbonate form in a 125-ml round-bottomed flask was added carefully a solution of 0.385 g (1.67 mmol) of N-*t*-butyloxycarbonyl-ϵ-aminocaproic acid in 10 ml of dioxane. The mixture was gently mixed by swirling, then evaporated with the aid of a rotary evaporator at 30–35° (35–10 mm) for 1 hr. The resin (1.13 g) was heated *in vacuo* at 80° for 4 hr. The resin product was triturated with warm dioxane, removed by filtration, further washed with dioxane, and dried, yielding 0.948 g. Evaporation of the filtrate and washings left 0.040 g (0.17 mol) of unchanged acid making the net mmoles of acid used 1.50. Thus the mmoles of N-*t*-butyloxycarbonyl-ϵ-aminocaproic acid per gram of resin = 1.58. The esterification reaction was shown to be complete (see below) in the usual manner. (Found: N, 2.1; S, 1.1.)

Example 5.—To 11.69 g (6.77 mequiv of HCO_3^-) of tetramethylene(arylmethylene)sulfoniumstyrene-2% divinylbenzene copolymer resin bicarbonate form in a 250-ml round-bottomed flask was added a solution of 2.22 g (7.43 mmol) of N-*o*-nitrophenylsulfenyl-L-glutamine in 150 ml of dioxane. This mixture was swirled gently and evaporated with the aid of a rotary evaporator at 35–40° (45–10 mm) for 30 min and at 40° (≤1 mm) for 45 min. The yellow colored resin (5.73 g) was then heated at 80° *in vacuo* for 4 hr, cooled (wt 4.55 g), swirled with methylene chloride, collected on a filter, washed successively with ethyl acetate and acetic acid, and dried *in vacuo* at 80° for 4 hr, resin dry wt 4.1 g. Evaporation of the combined wash filtrates left 0.30 g of unidentified semisolid material.

Infrared (Nujol mull) analysis of the resin product showed strong ester and primary amide carbonyl absorptions at *ca.* 1735 and 1670 cm^{-1}, respectively. Absorptions for NH_2 were observed at *ca.* 3470 and 3300 cm^{-1}. There was no apparent absorption for $-C\equiv N$ which would have been formed by dehydration of the $-CONH_2$ function of the glutamine. (Found: N, 5.1; S, 5.2, corresponding to *ca.* 1.21 mmol of N-NPS-glutamine per gram of resin ester.)

Example 6.—To a suspension of 0.990 g (0.72 mequiv of CO_3^{2-}) of dimethyl(arylmethylene)sulfoniumstyrene-2% divinylbenzene copolymer resin carbonate form in 10 ml of dioxane was 0.281 g (1.03 mmol) of N-*o*-nitrophenylsulfenyl-L-threonine. After mixing, the mixture was evaporated as described in example 5. The resin, 0.536 g, was then heated *in vacuo* over solid potassium hydroxide for 5 hr at 80°. When cooled it was swirled in warm ethyl acetate, filtered, further washed with acetic acid and methylene chloride, and dried at 80° for 3 hr *in vacuo*. When cooled, a 0.368-g portion of the resin (0.448 g) was treated with a 1:1 solution of 1 *N* HCl in acetic acid and chloroform for 15 min to remove the N-*o*-nitrophenylsulfenyl protective group. The mixture was filtered and washed successively with acetic acid, chloroform, and three times with ethanol and dried over KOH *in vacuo*, resin wt 0.306 g. A 0.289-g portion of this was suspended in 20 ml of anhydrous trifluoroacetic acid and dry hydrogen bromide was bubbled into the suspension (protected from atmospheric moisture) to decouple threonine from the resin. After 90 min, the mixture was filtered and the resin washed twice with trifluoroacetic acid. The filtrate and washings were evaporated, the residue was diluted to 10 ml, and an aliquot was analyzed by the ninhydrin method for threonine. (Found: 5.02 mg of threonine/ml of solution equivalent to a total of 0.547 mmol or an ester conversion of 76%; mmoles of threonine per gram of HCl-treated resins = 1.45.) Infrared analysis (Nujol) on the HCl-treated resin showed ester carbonyl absorption at 1747 cm^{-1} and hydroxyl absorption at 3350 cm^{-1}.

(31) This procedure which omits the filtration step before drying proved excellent for ϵ-aminocaproic acid and L-leucine esterification reactions. These acids consistently showed poor stoichiometry in their reactions with the basic sulfonium resins in the procedures in examples 1–3, the uptake of acids being *ca.* 83–85%.

Example 7.—The first part of this reaction was conducted as described in example 2 using 3.088 g (1.72 mequiv of HCO_3^-) of dimethyl(arylmethylene)sulfoniumstyrene–2% divinylbenzene copolymer resin bicarbonate form and 0.403 g (1.86 mmol) of N-*t*-butyloxycarbonyl-L-valine. There was recovered 0.030 g (0.14 mmol) of unchanged acid. After the drying period the resin was transferred to a 100-ml round-bottomed flask, covered with *ca.* 30 ml of benzene. The mixture was stirred and refluxed; a Dean–Stark water trap containing *ca.* 0.5 ml of potassium chloride crystals was used to remove final residual water. After 4.5 hr of reflux the resin was collected on a filter, washed, and dried, wt 1.59. Evaporation of the mother liquor and wash left 0.023 g (0.12 mol) of N-*t*-butyloxycarbonyl-L-valine methyl ester.

Treatment of a 0.285-g sample with 1 *N* HCl in acetic acid (see below) showed the esterification reaction to be 99% complete.

The conversion of N-*t*-butyloxycarbonyl-L-valine to resin benzyl ester was 93%, to methyl ester 7%; the mmoles of N-*t*-butyloxycarbonyl-L-valine per gram of resin ester was 1.01.

Example 8.—To 2.26 g (1.56 mequiv of HCO_3^-) of diethyl(arylmethylene)sulfoniumstyrene–2% divinylbenzene copolymer resin bicarbonate form was added a solution of 0.336 g (1.56 mmol) of N-*t*-butyloxycarbonyl-L-proline in 10 ml of dioxane. After carefully mixing by swirling, the mixture was evaporated as described previously and heated *in vacuo* at 80–88° for 4.5 hr. The beads at room temperature, 1.05 g, were washed well with methylene chloride and dried. Esterification was shown to be complete as previously with HCl in acetic acid. Evaporation of the wash left 0.014 g (~0.06 mmol) of an oil, the infrared spectrum of which showed it to be N-*t*-butyloxycarbonyl-L-proline ethyl ester, ν ($CHCl_3$) 1700 [–NHC(=O)–] and 1750 cm^{-1} [–C(=O)OEt]. Conversion of N-*t*-butyloxycarbonyl-L-proline to resin benzyl ester was 96%, to ethyl ester 4%; the mmoles of acid per gram of resin ester was 1.43.

Determination of Completeness of Esterification Reactions.—The following procedure was followed to determine the completeness of esterification reactions of N-*t*-butyloxycarbonylamino acids and sulfonium resin.

A weighed sample (150–350 mg) of resin ester product was placed in a small sintered-glass funnel (10 × 27 mm) swelled and washed briefly with methylene chloride, the wash being discarded. The resin was then mixed with 0.5–1 ml of 1 *N* HCl in acetic acid. After several minutes the HCl–acetic acid was removed by suction and the resin was washed with about 1 ml each of acetic acid and water. (In this step any unchanged carboxylate anion is converted into the free acid with concomitant removal of the *t*-butyloxycarbonyl protective group.) The filtrate and washings were then evaporated *in vacuo* and the amount of residue (amino acid hydrochloride) was determined by weight or by ninhydrin analysis; from this quantity, the sample weight, the weight of the resin batch, and the total quantity of N-*t*-butyloxycarbonyl amino acid associated with the resin batch, per cent esterification, was calculated. An example of this procedure follows.

A solution of 0.515 g (2.37 mmol) of N-*t*-butyloxycarbonyl-L-valine in 10 ml of dioxane was mixed with a suspension of 3.43 g (2.20 mequiv) of dimethyl(arylmethylene)sulfoniumstyrene–2% divinylbenzene copolymer resin as described previously. The resin mixture was filtered and the resin was washed three times with dioxane (recovered 0.049 g, 0.23 mmol of acid from filtrate and wash). A 0.351-g sample of the wet resin (5.096 g) was treated as described above with HCl in acetic acid. The residue after evaporation of the filtrate and washings was diluted to 5 ml with water and the valine hydrochloride content was determined colorimetrically by the ninhydrin method and found to be 0.13 mmol, equivalent to 1.89 mmol for the entire resin batch. The net mmoles of N-*t*-butyloxycarbonyl-L-valine associated with resin were 2.14; therefore 88% of the theoretical amount of unchanged acid anion, assuming no esterification, was removed from the resin.

Esterification of N-*t*-Butyloxycarbonyl-L-isoleucine.—This esterification was conducted essentially as described in example 4 above using 3.17 g (1.71 mequiv of HCO_3^-) of dimethyl(arylmethylene)sulfoniumstyrene–2% divinylbenzene copolymer resin bicarbonate form and 0.432 g (1.80 mmol) of N-*t*-butyloxycarbonyl-L-isoleucine hemihydrate in 25 ml of dioxane. After heating to effect esterification, the resin, 1.792 g, was washed well with methylene chloride (to remove unchanged acid and N-*t*-butyloxycarbonyl-L-isoleucine methyl ester) and dried, 1.687 g. Evaporation of the wash solution left 80 mg of residue. This was redissolved in methylene chloride, extracted with saturated bicarbonate solution, washed with water, and dried (magnesium sulfate) to yield 50 mg (~0.2 mmol, ~12%) of crude N-*t*-butyloxycarbonyl-L-isoleucine methyl ester [ν (film) 1760 (ester >C=O), 1730 (urethan >C=O), and 3400 cm^{-1} (N–H)].

The material extracted was presumed to be unchanged acid, *ca.* 30 mg (~0.13 mmol). Computing as previously, the mmoles of N-*t*-butyloxycarbonyl-L-isoleucine per gram of resin = 0.87. A sample of the resin ester was suspended in trifluoroacetic acid and treated with anhydrous hydrogen bromide to decouple the amino acid from the resin. After filtration of the suspension and evaporation of the filtrate the residue was dissolved in water and applied to the long column of a Phoenix automatic recording amino acid analyzer. No peak for D-alloisoleucine could be detected in the chromatogram indicating that, at least within 1%, no racemization of N-*t*-butyloxycarbonyl-L-isoleucine had occurred during the esterification reaction.[32]

Esterification of Dimethyl(arylmethylene)sulfonium- (Chloride) styrene–2% Divinylbenzene Copolymer Resin with N-*t*-Butyloxycarbonyl-L-valine.—To a solution of 2.2 g (9.75 mmol) of N-*t*-butyloxycarbonyl-L-valine, 1.23 ml (8.8 mmol) in 20 ml of ethanol, was added 5.0 g [5.70 mmol of dimethyl(arylmethylene)sulfonium chloride, $-CH_2S^+Me_2$, and 0.70 mmol of arylmethylene chloride, $-CH_2Cl$] of dry sulfonium resin.[33] This suspension was stirred and heated at reflux for 48 hr. The mixture was filtered and the resin collected was washed with ethanol, water, and methanol and dried to yield 5.09 g of resin ester. A portion, 4.96 g, of the resin was suspended in 20 ml of trifluoroacetic acid and treated with anhydrous hydrogen bromide for 1 hr to decouple the amino acid from the resin. The resin was collected on a filter and washed twice with trifluoroacetic acid. The combined filtrate and washings were evaporated *in vacuo* on a rotary evaporator at room temperature and the residue was further dried *in vacuo* over solid KOH. Ninhydrin analysis of the residue, 0.913 g, showed it to contain 0.477 g of L-valine, equal to a total yield of 0.488 g (4.18 mmol) or 0.82 mmol/g of substituted resin ester. The yield was 65% based on the total number of replaceable groups or 73% based only on the sulfonium group.

Esterification of chloromethylated trimethyl(arylmethylene)-ammonium- (chloride) styrene–2% divinylbenzene copolymer resin with N-*t*-butyloxycarbonyl-L-valine was carried out as described in the previous experiment using 2.17 (10 mmol) of N-*t*-butyloxycarbonyl-L-valine, 1.26 ml (9 mmol) of triethylamine, 20 ml of ethanol, and 3.85 g (9.5 mmol of arylmethylene chloride, $-CH_2Cl$) of chloromethylated trimethyl(arylmethylene)-ammonium- (chloride) styrene–2% divinylbenzene copolymer resin. The resin ester product (4.31 g) was found to contain 1.18 mmol of valine/g of resin ester. The yield was 57%.

Dimethyl(arylmethylene)sulfonium- (Chloride) trimethyl(arylmethylene)ammonium- (Chloride) styrene–2% Divinylbenzene Copolymer Resin. Preparation of and Esterification with *t*-BOC-amino Acids.—A suspension of 10 g [24.7 mmol of arylmethylene chloride, $-CH_2Cl$, 16 mmol of $-CH_2N^+(CH_3)_3Cl^-$] of chloromethylated trimethyl(arylmethylene)ammonium- (chloride) styrene–2% divinylbenzene copolymer resin, 16 ml (218 mmol) of methyl sulfide, 30 ml each of methylene chloride and methanol, and 40 ml of water was stirred at room temperature for 3 days whereupon additional methyl sulfide, 10 ml, was added and the suspension was heated at reflux for 1 day, stirring continued. The resin was collected on sintered glass and washed successively with dioxane–water (3:1), dioxane–2 *N* HCl (3:1), water–methanol, methanol, and ether and dried at room temperature, yield 11.2 g. Analysis of the resin product showed it to contain 0.29 mmol/g of covalent chloride making the conversion of $-CH_2Cl$ to $-CH_2S^+Me_2Cl^-$ 87% (assuming no hydrolysis, rearrangement, etc.). The total ionic content was 3.17 mmol/g ≅ 1.43 mmol/g of $-CH_2N^+Me_3Cl^-$ and 1.74 mmol/g of $-CH_2S^+Me_2Cl^-$. These calculations assume no material losses.

This resin was esterified with N-*t*-BOC-amino acids (glycine and L-valine) using triethylamine and ethanol as described in preceding experiments; the results are summarized in Table I.

Acknowledgment.—We wish to acknowledge a number of personnel of the Physical Research, Plastics

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(33) Dry sulfonium resin is less stable than the hydrated form and tends to revert back to chloromethylated resin and methyl sulfide.

Department Research, Ethylene Research, and Edgar C. Britton Research Laboratories for helpful discussion and suggestions during the course of this work. We also wish to thank Professor M. Calvin for his initial suggestion on the use of sulfonium compounds and Mr. John Kadlabitsky for laboratory assistance.

Heterocycles from Keto Acids with Amino Alcohols, Diamines, and Mercaptoamines

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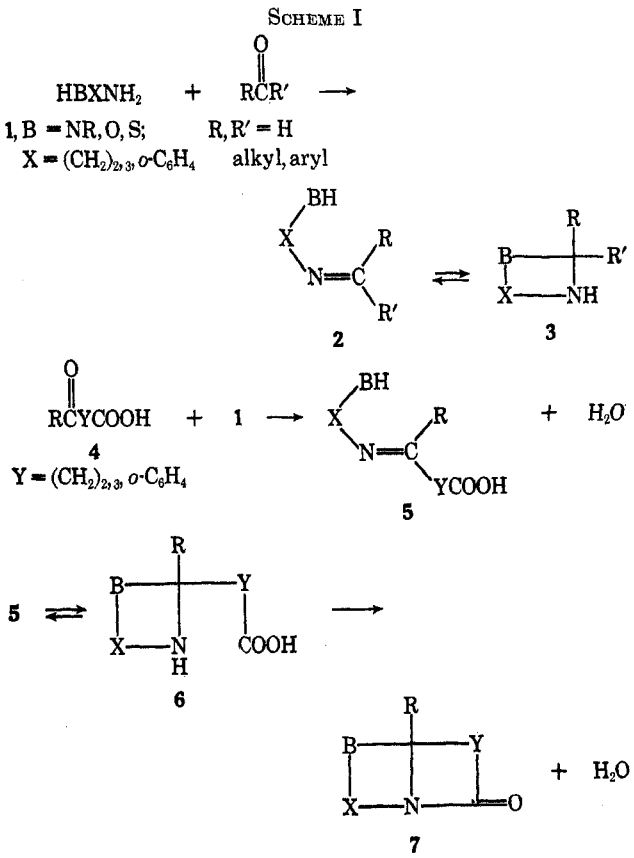
The condensation of a γ- or δ-keto acid with a 1,2-, 1,3-, or 1,4-amino alcohol, diamine, or mercaptoamine gave products containing lactams with an additional N-, O-, or S-containing ring fused on the a face of the lactam ring. The nuclear magnetic resonance of several ring systems gave an unusually low field proton signal that was assigned to the H_B proton of CH_AH_BNCO (21). The occurrence of this low-field proton was dependent on the ring size and heteroatom present in ring B of 21.

The reaction of a 1,2- or a 1,3-amino alcohol,[1a,b] diamine,[1c,d] or mercaptoamine[1e] (1) with an aldehyde or ketone has found general synthetic application in the preparation of heterocyclic systems 3.[1] The formation of 3 arises from the intermediate Schiff base 2 which in some systems exists in a ring-chain tautomeric equilibrium[2] with 3. An extension of this synthesis to an aldehyde or keto acid 4 suggests that the expected Schiff base 5 can first cyclize to the amino acid 6 and then proceed to form a second ring (7) from the available amino and carboxyl groups (Scheme I).

At the time our work was initiated no systematic study of the synthesis of compounds 7 by this reaction had been carried out. Limited studies of 2-mercaptoethylamine,[3a-d] cysteine,[3a] *o*-phenylenediamine,[3e-g] 2-aminobenzylamine,[3f] δ,δ′-diamino-*o*-xylene,[3e] 1,8-naphthalenediamine,[3e] and 2,2′-diaminobiphenyl[3e] with selected oxo acids (4) gave the heterocyclic systems 7 and their dehydrogenated[3e,g] analogs. Sulkowski[4] reported that aliphatic diamines and *o*-phenylenediamines react with 2-aroylbenzoic acids[4a] and 3-aroylpropionic acids[4b] to give medium-sized heterocycles 8. Geigy[5] workers carried out similar reactions with diamines, aminothiols, and amino alcohols and have assigned the fused-ring structure 9 to these condensation products. Very recently two papers[6a,b] and a patent[6c] appeared that presented evidence to show that the products from 2-aroylbenzoic acids and ethylenediamines are 5H-imidazo[2,1-*a*]isoindol-5-ones (9) and not 2,5-benzodiazocin-1-ones (8) as reported earlier.[4a]

SCHEME I



In the present paper we report our findings on the type of product formed when an amino alcohol, a diamine, or a mercaptoamine is condensed with 2-benzoylbenzoic, 3-benzoylpropionic, or 4-benzoylbutyric acids.

(1) For a general survey, see (a) J. W. Cornforth in "Heterocyclic Compounds," Vol. 5, John Wiley & Sons, Inc., New York, N. Y., 1957, pp 391–395; (b) N. H. Cromwell, ref 1a, Vol. 6, pp 541–544; (c) E. S. Schipper and A. R. Day, ref 1a, Vol. 5, p 245; (d) G. W. Kenner and Sir A. Todd, ref 1a, Vol. 6, pp 314–316; (e) J. M. Sprague and A. H. Land, ref 1a, Vol. 5, pp 697–702; (f) J. W. Keana, S. B. Keana, and D. Beetham, *J. Amer. Chem. Soc.*, **89**, 3055 (1967).

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(5) J. R. Geigy, A.G. Belgian Patent 659,528 (Aug 10, 1965); *Chem. Abstr.*, **64**, 3545 (1966); Belgian Patent 659,530 (Aug 10, 1965); *Chem. Abstr.*, **64**, 6664 (1966), and Netherlands Patent Application 6,501,640 (Aug 12, 1965).

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