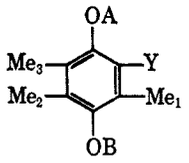
TABLE I
CHEMICAL SHIFTS OF THE METHYL GROUPS[k]



Compd	Y (τ)	A (τ)	B (τ)	Me1	Me2	Me3
I	H	H	H	7.98	7.83	7.83
II[a]	H (3.50)	CH_3 (6.25)	H	7.84	7.84	7.79
III[b]	H (3.65)	CH_3 (6.33)	CH_3 (6.48)	7.95	7.88	7.81
IV[c]	H	CH_3 (6.31)	CH_3 (6.31)	7.88		7.88
V[d]	CH_3 (7.89)	CH_3 (6.46)	CH_3 (6.46)	7.89	7.89	7.89
VI[e]	H	$COCH_3$ (7.68)	$COCH_3$ (7.65)	7.88	7.93	7.93
VII[f]	CH_3 (7.96)	$COCH_3$ (7.67)	$COCH_3$ (7.67)	7.96	7.96	7.96
VIII[g]	$CH=CH_2$	CH_3 (6.45)	CH_3 (6.50)	7.84	7.90	7.90
IX[c,g]	$CH=CH_2$	CH_3 (6.47)	CH_3 (6.34)	7.81		7.90
X	Br	CH_3 (6.28)	H	7.68	7.89	7.78
XI[h]	CH_2Cl (5.31)	CH_3 (6.26)	H	7.86	7.86	7.77
XII[i]	$CH=CH_2$	CH_3 (6.41)	H	7.82	7.82	7.88
XIII	CH_2Cl (5.27)	CH_3 (6.20)	$COCH_3$ (7.68)	7.82	7.95	7.82
XIV[j]	CH_2Cl (5.30)	H	$COCH_3$ (7.66)	7.95	7.95	7.84
XV	CH_2Cl (5.29)	$COCH_3$ (7.67)	H	7.90	7.85	7.95
XVI	Br	CH_3 (6.24)	$COCH_3$ (7.66)	7.76	7.98	7.76
XVII[i]	$CH=CH_2$	CH_3 (6.39)	$COCH_3$ (7.69)	7.81	7.96	7.89
XVIII	CH_2OCOCH_3 (4.77) (7.93)	CH_3 (6.29)	$COCH_3$ (7.66)	7.89	7.93	7.78

[a] Reference 10. [b] L. I. Smith, *J. Am. Chem. Soc.*, **56**, 472 (1934). [c] L. I. Smith and J. W. Opie, *J. Org. Chem.*, **6**, 427 (1941). [d] L. I. Smith and H. C. Miller, *J. Am. Chem. Soc.*, **64**, 440 (1942). [e] R. Nietzki and J. Schneider, *Ber.*, **27**, 1426 (1894). [f] L. Rügheimer and M. Hankel, *ibid.*, **29**, 2171 (1896). [g] K. A. Kun and H. G. Cassidy, *J. Polym. Sci.*, **56**, 83 (1962) [h] Reference 5. [i] N. Nakabayashi, G. Wegner, and H. G. Cassidy, *J. Polym. Sci., A-1*, **6**, 869 (1968). [j] Reference 3. [k] All data taken in $CDCl_3$ on a Varian Associates Model A-60 spectrometer.

acetate, and 7.92–7.95 to the methyl of the acetates on the side chains.

Experimental Section[9]

3-Bromo-4-methoxy-2,5,6-trimethylphenol (X).—To a solution of 33.2 g (0.20 mol) of 4-methoxy-2,3,6-trimethylphenol[10] (II) in 400 ml of carbon disulfide was added 32.0 g (0.20 mol) of bromine gradually with stirring. After a 1-hr reaction at room temperature, the solvent was taken off and the residue was recrystallized from *n*-heptane to give 36.6 g (74.8%) of X, mp 119.5–120°. *Anal.* Calcd for $C_{10}H_{13}BrO_2$: C, 49.00; H, 5.34. Found: C, 48.97; H, 5.39.

3-Acetoxy-6-hydroxy-2,4,5-trimethylbenzyl Chloride (XIV) and 2-Acetoxy-5-hydroxy-3,4,6-trimethylbenzyl Chloride (XV).—The preparation of XIV had been reported.[3] By following those directions, omitting the Norit treatment, 9.7 g of XIV (mp 149–151°, recrystallized from ether–*n*-hexane) and 5.0 g of XV (mp 149–150° from ether) were obtained. The latter was less soluble in ether than the former. The mixture melting point of XIV and XV was 147–150°.

3-Acetoxy-6-methoxy-2,4,5-trimethylbenzyl Chloride (XIII).—To a stirred and cooled solution of 4.3 g (0.02 mol) of XI and 2.4 g (0.03 mol) of acetyl chloride in 30 ml of THF and 30 ml of ether was added 3.0 g (0.03 mol) of triethylamine in 10 ml of ether over a period of 10 min. Two hours later triethylamine hydrogen chloride was filtered off, the organic solvents were evaporated to dryness, and the residue was recrystallized from *n*-hexane. The yield of XIII was 4.6 g (90.1%), mp 99–100°. *Anal.* Calcd for $C_{13}H_{17}ClO_3$: C, 60.83; H, 6.67. Found: C, 60.86; H, 6.48.

3-Acetoxy-6-methoxy-2,4,5-trimethylbromobenzene (XVI).—The compound was prepared quantitatively from X, acetyl chloride, and triethylamine. The purification was carried out from *n*-hexane; mp 97–98°. *Anal.* Calcd for $C_{12}H_{15}BrO_3$: C, 50.19; H, 5.26. Found: C, 50.20; H, 5.31.

3-Acetoxy-6-methoxy-2,4,5-trimethylbenzyl Acetate (XVIII).—A mixture of 4.3 g (0.02 mol) of XI and 30 ml of acetic anhydride was refluxed in the presence of one drop of concentrated sulfuric acid for 1 hr. The anhydride was evaporated and the brown viscous residue was recrystallized from *n*-hexane to give 2.9 g of small white needles, mp 62–65°. This is a 50% mixture of XIII and XVIII, on the basis of the nmr spectra (compared at τ 4.80, 5.28 and 6.19, 6.28, respectively). Fractional crystallization, twice, from a rather large amount of *n*-hexane gave pure XVIII, mp 88–89°. The mixture mp of XIII and XVIII was 62–65°. Compound XVIII could be purified by sublimation. The ir spectrum of XVIII showed two carbonyl bands at 1754 and 1731 cm^{-1}. *Anal.* Calcd for $C_{15}H_{20}O_5$ (XVIII): C, 64.27; H, 7.19. Found: C, 64.11; H, 7.12.

Compound XVIII was also prepared from XIII by refluxing 1 hr with acetic anhydride and 1 drop of concentrated sulfuric acid; yield 55%.

(9) Methods for new compounds only are given; see Table I. Melting points are all corrected.

(10) W. John and F. H. Rathmann, *Ber.*, **73**, 995 (1940).

Registry No.—I, 700-13-0; X, 18910-32-2; XIII, 18910-33-3; XIV, 18910-34-4; XV, 18910-35-5; XVI, 18910-36-6; XVIII, 18910-37-7.

Acknowledgment.—We are glad to acknowledge that this work was supported by PHS Research Grant GM 10864, Research Grants Branch, National Institute of General Medical Sciences, Public Health Service.

Formation of Benzyne by the Reaction of *o*-Phenylene Carbonate with Neutral Trivalent Phosphorus Nucleophiles

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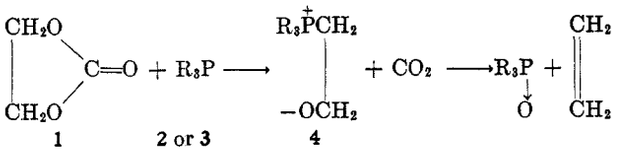
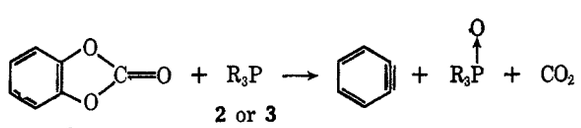
Keough and Grayson have shown that the reaction of ethylene carbonate (**1**) with triphenylphosphine (**2**) at

elevated temperatures results in oxidation of **2** and the formation of carbon dioxide and ethylene.[1] Olefin formation also occurs with other (propylene, phenyl-



ethylene) cyclic carbonates and the reaction is subject to catalysis by metallic copper and proceeds more reasily with the stronger nucleophile tri-*n*-butylphosphine (**3**). The reaction was thought to proceed by attack of **2** or **3** at an ester carbon of **1** to yield betaine **4** and carbon dioxide, followed by decomposition of **4** through a typical Wittig four-membered transition state to yield the phosphine oxide and ethylene. In the case of the less reactive phenylethylene carbonate, a mechanism involving attack of **2** or **3** at the carbonyl carbon to yield the betaine, $R_3P^+COOCH_2CH(C_6H_5)O^-$ (**5**), was considered feasible; **5** (or its uncharged cyclic form) could then collapse in a concerted manner to yield the observed products. Based on these mechansitic postulations, an examination of the reactions of **2** and **3** with *o*-phenylene carbonate (**6**) appeared to be of interest. If such reactions were to parallel the reaction of **1**, benzyne should be produced as a reactive intermediate and the over-all process could potentially serve as a



method for the production of this useful species in in aprotic media.[2]

No reaction was observed when equimolar quantities of *o*-phenylene carbonate (**6**) and triphenylphosphine (**2**) as a melt or in diphenyl ether or Nujol solutions were held at 250° for 24 hr; similar attempted reactions of **2** and **6** in refluxing diglyme solution for 96–108 hr also failed. No effect was observed on the addition of metallic copper dust. Reaction of equimolar quantities of **6** and tri-*n*-butylphosphine (**3**) in the presence of copper dust in an evacuated sealed tube at 190–200° for 24 hr did, however, result in consumption of **6** (disappearance of ν_{CO}) and the formation of tri-*n*-butylphosphine oxide. Glpc analysis of the products showed the presence of *ca.* 4% of the phosphine oxide and *ca.* 3% of triphenylene, the normal trimerization product of benzyne.[2b] When the same reaction was conducted in the presence of furan and anthracene in an attempt to trap the benzyne formed, only trace amounts of the Diels–Alder adducts, 1,4-dihydronaphthalene 1,4-endoxide and triptycene,[2c] respectively, could be detected. However, a similar reaction of equimolar quantities of **3**, **6**, and the more effective benzyne trapping agent, tetracyclone,[2c,3] led to the complete consumption of **6** and the formation of the Diels–Alder adduct, 1,2,3,4-tetraphenylnaphthalene (2.5%). Corresponding reactions of **6** with excess or equimolar quantities of trimethyl or triethyl (**7**) phosphite in the presence of copper dust led to the consumption of **6**, but no triphenylene could be detected nor could a successful interception with tetracyclone be achieved. In all of the reactions with **3** and the trialkyl phosphites, an appreciable quantity of higher boiling materials was formed.

The results of the reactions of **6** with **3** and with the trialkyl phosphites indicate that benzyne is formed as an intermediate, although the yields of derived products are quite low. In view of the complete consumption of **6** in these reactions, the low yield of benzyne products must be due to either ineffective trapping or alternative modes of consumption of benzyne or reactions of **6** which do not produce benzyne. Borowitz and Anschel[4] have shown that fluorenones react with **3** and trialkyl phosphites to yield a variety of high molecular weight condensation products. It is probable that a corresponding reaction with tetracyclone consumes that reagent and accounts for the inefficient interception of benzyne. In a control experiment, it was shown that **7** and tetracyclone react rapidly at 150°; tetracyclone is completely consumed in 30 min at this temperature.

A second likely reason for the low yields of benzyne-related products in these reactions is the reactivity of benzyne toward neutral trivalent phosphorus nucleophiles. A number of investigators have shown that such nucleophiles react with benzyne to yield dipolar structures which can be trapped to produce tetracovalent phosphorus products.[2d] Thus, Seyferth and Burlitch[5] have shown that benzyne and diphenylmethylphosphine react to yield a dipolar product which undergoes proton transfer to give the ylide $(C_6H_5)_3P{=}CH_2$. Similarily, it has been shown that the dipolar adduct of triethyl phosphite (**7**) with benzyne undergoes, in the absence of good nucleophiles, a β-elimination process to yield diethyl phenylphosphonate (**8**) and ethylene.[6] Consumption of benzyne in the phenylene carbonate reaction by these processes could account for the low yields of benzyne products.

In order to substantiate this postulation, the thermal (200°, 24 hr) reactions of excess **3** and **7** with **6** in the presence of copper dust were examined. In each case, carbonate **6** was completely consumed with the production of 1 equiv of the oxide of **3** or **7**. The reaction mixture from **3** and **6** was treated with excess ethereal hydrogen bromide to produce tri-*n*-butylphenylphosphonium bromide (**9**, 14%). The reaction of benzyne with **3** would be expected to yield the ylide $C_6H_5(n\text{-}C_4H_9)_2P{=}CHCH_2CH_2CH_3$, in analogy with the diphenylmethylphosphine reaction;[5] reaction of this ylide with hydrogen bromide would produce **9**. Glpc analysis of the reaction mixture of **6** and **7** showed the presence of diethyl phenylphosphonate (**8**, 22%), the expected[6] reaction product of benzyne and **7**. Product **8** was isolated in 18% yield. A typical procedure for this reaction is given in the Experimental Section.

Although the results of this study indicate that benzyne can be generated in aprotic media by the

(1) P. T. Keough and M. Grayson, *J. Org. Chem.*, **27**, 1817 (1962).

(2) For a summary of the chemistry of benzyne and the methods available for its generation, see R. W. Hoffmann, "Dehydrobenzene and Cycloalkynes," Academic Press, New York, N. Y. 1967: (a) pp 9–87; (b) p 110; (c) pp 208–237; (d) pp 168–171.

(3) E. LeGoff, *J. Amer. Chem. Soc.*, **84**, 3786 (1962).

(4) I. J. Borowitz and M. Anschel, *Tetrahedron Lett.*, 1517 (1967).

(5) D. Seyferth and J. M. Burlitch, *J. Org. Chem.*, **28**, 2463 (1963).

(6) C. E. Griffin and N. T. Castellucci, *ibid.*, **26**, 629 (1961); V. A. Notaro, Ph.D. Thesis, University of Pittsburgh, 1965.

reactions of *o*-phenylene carbonate with tri-*n*-butylphosphine and triethyl phosphite, the subsequent reactions of the intermediate with the nucleophiles precludes the use of this procedure for the generation of benzyne for reaction with other species.

Experimental Section

Reaction of *o*-Phenylene Carbonate (6) with Triethyl Phosphite (7).—A mixture of 6.80 g (50 mmol) of **6**, 31.5 g (250 mmol) of **7** and 0.1 g of copper dust was placed in a 100-ml Pyrex tube, which was evacuated and sealed. The reaction mixture was held at 200–205° in an oil bath for 24 hr and allowed to cool to room temperature. Glpc analysis[7] of the reaction mixture showed the presence of **7** (5%), triethyl phosphate (101%), diethyl phenylphosphonate (**8**, 22%), and triphenylene (3%) (yields are based on **6**). No unreacted **6** was detected; a large amount of diethyl ethylphosphonate, the thermal isomerization product of **7**, was present. Distillation of the reaction mixture led to the isolation of **7**, triethyl phosphate, and **8** (1.93 g, 18%), identified by comparison with an authentic sample.[7]

Registry No.—Benzyne, 462-80-6; **6**, 2171-74-6; **7**, 122-52-1.

Acknowledgment.—This study was supported in part by a grant from the Gulf Oil Foundation.

(7) For columns and conditions, see J. B. Plumb and C. E. Griffin, *J. Org. Chem.*, **28**, 2908 (1963); J. B. Plumb, R. Obrycki, and C. E. Griffin, *ibid.*, **31**, 2455 (1966).

The Synthesis of Peptides in Aqueous Medium. VI.[1] The Synthesis of an Unsymmetrical Cystine Peptide Fragment of Insulin

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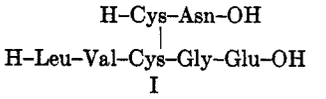
It has been reported[2] that enzymatic degradation of insulin with the enzymes of rat adipose tissue gives peptide fragments which show a high degree of insulin activity. One of the fragments reported to possess insulin activity is the sequence A chain 20–21 coupled through a disulfide bond to B chain 17–21 (I). To determine in an unequivocal manner whether this small part of the insulin molecule is indeed biologically active, we undertook the synthesis of I.



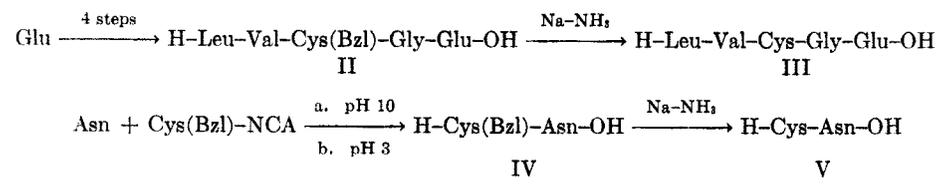
I

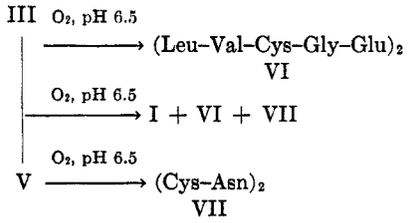
A number of methods[3] for the synthesis of unsymmetrical disulfides have been reported. The least attractive of these methods would appear to be the direct oxidation of a mixture of the two cysteine peptides, since a mixture of three peptides would be expected.[4] However, we felt that the ready availability of the dipeptide Cys–Asn would allow its use in large excess thus permitting the most effective utilization of the pentapeptide Leu–Val–Cys–Gly–Glu. We, therefore, undertook the preparation of the two necessary peptides as outlined in Scheme I.

Both peptides were prepared by the N-carboxyanhydride (NCA) procedure.[5,6] The preparation of II was carried out in four steps without isolation of intermediates, but each step was monitored by thin layer chromatography. In the preparation of Gly–Glu, we replaced the NCA of glycine by 2,5-thiazolidinedione which has been shown to afford significantly higher yields than the N-carboxyanhydride.[1] On acidification to pH 3 to dethiocarboxylate the initially formed thiocarbamoyl dipeptide, the reaction mixture was shown to contain no detectable glutamic acid and only a trace of glycine by tlc. Subsequent addition of the NCA's of S-benzylcysteine, valine, and leucine, respectively, gave II. On the final acidification (after the addition of the NCA of leucine) the crystalline product separated from the reaction medium. This material was not completely pure (minor peptide impurities were observed on tlc). The amino acid analysis was consistent with II and the material in this form was satisfactory for the subsequent preparation of pure I and VI.

The dipeptide IV was prepared by the action of S-benzylcysteine N-carboxyanhydride on asparagine. The crystalline product was identified by elemental analysis and base titration. The S-benzyl blocking groups of II and IV were removed by the action of sodium in liquid ammonia to give III[7] and V, respectively. An aliquot of each of these in aqueous solution was air oxidized at pH 6.5 to give the expected cystine peptides VI and VII, respectively. When a mixture of

SCHEME I





(1) Part V: R. S. Dewey, E. F. Schoenewaldt, H. Joshua, W. J. Paleveda, H. Schwam, H. Barkemeyer, B. H. Arison, D. F. Veber, R. G. Denkewalter, and R. Hirschmann, *J. Am. Chem. Soc.*, **90**, 3254 (1968).

(2) D. Rudman, L. A. Garcia, M. DiGirolamo and P. W. Shank, *J. Clin. Invest.*, **44**, 1093 (1965); *Endocrinology*, **78**, 169 (1966).

(3) E. Schröder and Lubke, "The Peptides," translated by E. Gross, Vol. 1, Academic Press, New York, N. Y., 1965, pp 236–239.

(4) A. H. Livermore and E. C. Muecke, *Nature*, **173**, 265 (1954).

(5) R. G. Denkewalter, H. Schwam, R. G. Strachan, T. E. Beesley, D. F. Veber, E. F. Schoenewaldt, H. Barkemeyer, W. J. Paleveda, T. A. Jacob, and R. Hirschmann, *J. Amer. Chem. Soc.*, **88**, 3163 (1966).